TECHNICAL INFORMATION

Biological Buffers and pH Control

All media used in tissue culture have as a basis a synthetic mixture of inorganic salts known as a 'physiological' or balanced salt solution. The functions of this salt solution in the medium are to maintain proper pH, maintain ideal osmotic pressure, and provide a source of energy. The growth of animal cells in a nutritionally complete tissue culture medium is usually optimal when the medium is buffered at a pH in the range of 7.2-7.4. To function most effectively, the pKa of the chosen buffer should be as close as possible to the required pH.

The most commonly used buffer in tissue culture media is sodium bicarbonate. However, this buffer has two important disadvantages. First, the pKa of sodium bicarbonate is 6.3 at 37°C which results in suboptimal buffering throughout the physiological pH range. Second, carbon dioxide is released in the atmosphere with a resulting increase in alkalinity, and the number of hydroxyl ions produced increases according to the amount of sodium bicarbonate added to the medium. It is possible to control this by artificially supplying carbon dioxide to the atmosphere and preventing the gas from leaving the liquid, thereby reducing the hydroxyl ion concentration in solution.

Balanced salt solutions can be divided into two types: those intended to equilibrate with air in a closed system at a low concentration of sodium bicarbonate (Hanks' Balanced Salt Solution) and those intended to equilibrate with a gaseous phase containing approximately 5% CO_2 at a higher concentration of sodium bicarbonate (Earle's Balanced Salt Solution). Earle's Balanced Salt Solution is a much better solution because it contains a greater amount of sodium bicarbonate, but it is more difficult to use since it requires a special gaseous mixture of 5% CO_2 with 95% air to be provided by the culture medium. If this procedure is not carried out, the pH increases rapidly at normal incubation temperatures. The purple color of the medium indicates that the pH has risen, and cell growth is inhibited.

An alternative method is to use a medium which produces sufficient buffering capacity but does not require 5% CO_2. In some cases, this can be achieved by using a medium containing Earle's salts but having the concentration of sodium bicarbonate reduced to 0.85 g/liter. An entirely different approach was devised by Leibovitz (1963). He utilized the buffering capacity of free base amino acids, omitted sodium bicarbonate, substituted galactose for glucose and added pyruvate. The pH of his L-15 medium is approximately 7.8 which is higher than that of most other media. Since there is no production or loss of CO_2, the pH will not rise further. This medium makes possible the growth of cells in open culture vessels without regard to the CO_2 content of the atmosphere.

Attempts have been made in recent years to find the most suitable buffer. The most commonly utilized alternative to bicarbonate is HEPES buffer which was first described by Good, et al. (1966). This acts as a zwitterion and has proved superior to conventional buffers in comparative biological assays with cell-free preparations. It has many properties which make it ideal as a buffer to tissue culture media, principally in that it does not require an enriched atmosphere to maintain the correct pH. HEPES does not bind divalent cations and is soluble to the extent of 2.25 M at 0 degrees. Note: since the DpKa/°C of -0.014, the pH reading recorded in a HEPES buffered medium will vary inversely with the temperature of the medium.

The following table gives expected pH levels at various temperatures:

Temperature °C	pH	Temperature °C	pH
5	7.58	23	7.47
15	7.56	24	7.46
16	7.55	25	7.44
17	7.54	26	7.43
18	7.53	27	7.42
19	7.52	28	7.41
20	7.50	29	7.40
21	7.44	30	7.38
22	7.48	37	7.30

HEPES is satisfactory as a buffer in tissue culture media for the growth of many different types of cells and viruses. It may exhibit toxicity at concentrations greater than 40 mM. Studies have indicated that 20 mM HEPES is the most satisfactory concentration for the buffer when both Hanks' and Earle's solutions are used. CO_2 incubators should not be used with media buffered solely with HEPES. ICN HEPES buffered liquid media are produced with a pH of 7.2-7.4 at 37°C. Powdered media are prepared so that a 1X solution will have a pH of 7.2-7.4 at 37°C without further adjustment.

Sodium bicarbonate should also be added as a nutritional requirement. It is recommended that the sodium bicarbonate concentration should not exceed 10 mM when the HEPES concentration is 20 mM. All single-strength (1X) liquid media contain either sodium bicarbonate or HEPES buffer or both. Ten times concentrated (10X) liquid media do not contain buffer and powdered media are either buffer free or contain HEPES buffer only. Whenever sodium bicarbonate is used to buffer tissue culture media at a concentration of greater than 1.0 g/liter, a CO_2 enriched atmosphere is required using a CO_2 incubator.

Mammalian cells can survive over a wide pH range (6.6-7.8), but as a rule, the optimal growth of cells is obtained at pH 7.2-7.4. It is undesirable to allow the pH to deviate outside the limits of pH 6.8-7.6. It should be remembered that no buffer is capable of holding the pH constant in a system in which acids or bases are being produced. Buffers only slow the rate of pH change. Cells in culture produce acidic products which act to lower the pH of the medium.

Most of the media utilize phosphates and the bicarbonate system to buffer the media. The bicarbonate ion can be converted to gaseous carbon dioxide and lost from the medium resulting in a rise in the pH. Carbon dioxide can be maintained by supplying a special gas phase or by sealing the vessel tightly so that the CO_2 produced by metabolic processes is retained in the vessel and re-absorbed by the medium.

Efforts to eliminate bicarbonate as a buffer system have been reported in medium SR1-8 and in medium L-15. Both media contain a phosphate buffer and increased amounts of amino acids to accomplish the buffering required.

A number of organic compounds have been described that have buffering capacity in the required range. HEPES will have a pronounced effect on the final pH. It is necessary to measure the pH at the temperature of use to determine the final pH due to the contribution by other buffers. HEPES buffer may be sterilized by steam and adjusted to the desired pH with sodium hydroxide. Concentrations of 10-25 mM have been employed with no apparent toxicity. When utilizing HEPES, ICN chooses a 20 or 25 mM concentration in the preparation of media unless it is otherwise specified. A mixture of acid form and base form HEPES is used in combination in most of ICN's media as this has been found to produce a more stable buffering system. Sodium chloride is reduced to keep the milliosmolality of the media containing both bicarbonate and HEPES in the range on non-HEPES containing media.

Powder Media Use

Since Swim and Parker demonstrated that powdered media can be prepared by ball-milling of the component chemicals, the use of powdered media has grown significantly. The use of a powdered medium allows the investigator uniformity that is not usually attainable from conventionally prepared liquid media in that a single batch of medium may be employed in long term or replicate experiments. Long term stability of powders and ease of storage are two of the advantages of powdered media often cited.

Autoclavable powdered media have not been employed routinely although the usefulness of such preparations is readily apparent. Succinic acid and sodium succinate are utilized to maintain the pH of 4.0-4.5 during autoclaving. Instructions for the preparation of both powdered media and Auto-Pow® (autoclavable powder) are as follows:

Powder Media Preparation

1. Measure approximately 10% less water (distilled or deionized) than the final volume of medium desired. Water should be room temperature (15-30°C).
2. Stir water and slowly add the powder. Rinse out the inside of the container to remove all traces of powder. Continue stirring until the powder has dissolved. Some media will not dissolve completely unless the pH is adjusted to neutral. For these, lower the pH to about 7.0 to facilitate solution formation.
3. Add the desired amount of sodium bicarbonate solution or powder and stir until dissolved. Do not over mix.
4. Dilute to the final volume and stir gently. Do not over mix.
5. Check and if necessary, adjust the pH using 1N hydrochloric acid or 1N sodium hydroxide. The pH of bicarbonate containing media will rise 0.2-0.3 units on filtration. This should be considered in adjusting the pH.
6. Sterilize immediately by membrane filtration using positive pressure. Nitrogen or air is recommended. Do Not Use Carbon Dioxide.
7. Aseptically dispense into sterile containers allowing only sufficient headspace for any necessary supplements.
8. Label and store at 2-8°C. Salt solutions may be stored at 15-30°C.

Auto-Pow® (Autoclavable Powder) Preparation

1. Measure out approximately 10% less purified water (distilled or deionized) than the final volume of medium desired. Water should be room temperature.
2. Stir the water and slowly add the powder. Rinse out the inside of the container to remove all traces of powder. Continue stirring until the powder has dissolved. NOTE: it is possible to weigh out the desired quantity of powder from a container using the weight noted in the product label. It is recommended, however, to use an entire container at once., in which case it is not necessary to weigh the powder.
3. Dilute with water to a volume equal to the final volume minus the amount of sodium bicarbonate solution to be added in step 5. Mix well and dispense into suitable containers. These can be the final containers provided sufficient space remains for any necessary supplements to be added after sterilization.
4. Autoclave at 121°C (1 Bar, 15 psi) for 15 minutes, with a slow exhaust cycle. Higher temperatures and/or longer times are not recommended.
5. After cooling, add sterile sodium bicarbonate solution as desired. Aseptically measure and if necessary adjust the pH using 1N hydrochloric acid.
6. Label and store at 2-8°C.

Media Concentrates

In producing 10X media and 10X balanced salt solutions, it is necessary to adjust the pH of the concentrate to achieve solubility of certain components. Therefore, when one is making a 1X solution from the 10X concentrate, it may be necessary to adjust the pH after the addition of sodium bicarbonate (if applicable) with sterile 1N sodium hydroxide or 1N hydrochloric acid to achieve the desired pH.

1X Media Preparation Procedures from Concentrate

1. Dilute 100 ml 10X media concentrate in 850-875 ml of sterile distilled, deionized, or RO water. Water should be depyrogenated, if possible.
2. Add recommended amount of sterile sodium bicarbonate solution.
3. Add appropriate amounts of other sterile ingredients such as L-glutamine, serum, etc.
4. Check pH and adjust with 1N sterile sodium hydroxide (or 1N hydrochloric acid) to achieve desired pH.
5. Add sterile water to liter, if necessary.

1X Balanced Salt Solution Preparation from Concentrate

1. Dilute 100 ml 10X Balanced Salt Solution concentrate in 850-900 ml of sterile distilled, deionized, or RO water. Water should be depyrogenated, if possible.
2. Add recommended amount of sterile sodium bicarbonate solution.
3. Check pH and adjust with 1N sterile sodium hydroxide (or 1N hydrochloric acid) to achieve desired pH.
4. Add sterile water to liter, if necessary.

Serum Handling Tips

At ICN, our goal is to provide maximum value of our products to all of our customers. To help ensure that you get satisfactory performance from your order of ICN CELLect™ serum, ICN recommends that you adhere to the following handling guidelines.

ICN CELLect™ serum should be inspected on arrival and stored at -20 to -10°C. Should the serum become partially thawed, we recommend that it be allowed to thaw completely at room temperature and then carefully mixed, avoiding frothing. The serum may be refrozen and stored at -20° to -10°C before use. In our experience, this will not decrease the growth promoting properties of the serum, and will help prevent precipitates and complexes from forming in the serum.

Avoid glass-to-glass and glass-to-metal contact. Most glass breakage is caused by bruising resulting from this type of contact. Avoid rapid temperature changes. **Never transfer a glass bottle directly from a freezer to a water bath.** Conversely, avoid transferring glass bottles directly from a water bath to a freezer. Always keep some space between serum bottles during storage.

Thawing Serum

When serum has been stored at -70 or -20°C, it should be allowed to equilibrate in a refrigerator at 2-6°C, preferably overnight. Placing a piece of cloth under the bottles during equilibration helps reduce glass breakage. Allow thawing to complete at room temperature. Thawing serum at high temperatures is not recommended. Periodic agitation during thawing is helpful. Turbidity or flocculent material may appear upon thawing or storage. This is caused by lipoproteins that may precipitate. This will not adversely affect the performance of the product.

Heat Inactivation

Complement is inherent in sera and can be inactivated by raising the temperature to 56°C. ICN offers FBS in a heat inactivated form upon request. However, it is a rather simple procedure to inactivate serum. Essentially, all that is needed to be done is to incubate the serum at 56°C in a water bath for 30 minutes. The serum must be gently swirled every 10 minutes during this incubation. Please be advised that heat inactivation may cause serum gelation. Sera with higher protein concentrations will gel more easily. Also, heat inactivation at higher temperatures or for longer periods of time may promote gelation.

Aseptic Technique

In cell culture, 70% of all problems are due to a lack of sound aseptic technique. Microorganisms which can result in contamination exist everywhere, from the surfaces of objects to the surrounding air. A conscious effort must be made to keep them out of the sterile environment.

Because cell culture techniques often entail several steps which can lead to contamination, cell culture media are often supplemented with antibiotics. Purchasing cell culture media from premium vendors such as ICN where quality assurance measures are rigorous will also greatly reduce the incidence of contamination. Nevertheless, this will not eliminate cell culture contamination resulting from poor sterile technique or antibiotic resistant mutants. Autoclaving will render pipettes, glassware and solutions sterile. Nutrient media cannot be autoclaved. The compounds in nutrient medium are destroyed by the heat generated during autoclaving. Media must, therefore, be sterilized by sterile filtration through filters small enough in pore size to hold back bacteria and mycoplasma.

Guidelines for Sterile Technique -

1. Wipe your work are clean with 70% ethanol prior to use. It may help to rinse your hands with ethanol as well.
2. Keep sterile flasks, bottles, and petri dishes covered until the instant to be used. Return any covers as soon as you are finished.
3. Sterile pipettes should NEVER be taken from the cylinder or wrapping until they are ready to be used. Keep your pipettes at your work area. Sterile pipettes DO NOT have to be flamed Pipetting your cells with a hot pipette will kill them.
4. When removing the cap from a bottle, flask, etc., keep it face down, grasping it with the little finger of your right hand. DO NOT place caps on the lab bench. Flame the lips of flasks, bottles and other cultureware before and after introduction of a pipette. DO NOT hold the opening straight into the air. If possible, tilt the container such that any falling microorganisms will land in the lip where they will be flamed upon closure.

5. Avoid talking, singing or whistling while performing sterile procedures as these generate aerosol contaminants.
6. NEVER PIPETTE BY MOUTH. Use a pi-pump or other device. Even though your pipettes are plugged, mycoplasma in your mouth can still pass through and become one of the largest problems in cell culture contamination.
7. DO NOT draw from a bottle more than once with the same pipette. Use a sterile pipette each and every time - especially when pipetting media!
8. Techniques should be performed as rapidly as possible to minimize contamination.

It is possible that you may find yourself performing a procedure not addressed by these sterile technique guidelines. Therefore, you must constantly be aware that microorganisms are everywhere and take proper steps to keep them out of your cultures. When first developing your aseptic technique, you must always think of sterility. Eventually, it will become second nature to you. Mastering good aseptic technique will save minimization of biohazard risk when infectious organisms or dangerous chemicals are being used.

Adaptation of Cells to Alternative Serum

The majority of established cell types currently cultured in media supplemented with FBS can be adapted to grow with ICN CELLect™ Iron-Supplemented Calf, Donor Calf or Newborn Bovine Serum. Complete adaptation may take between three to four subcultures and should be carried out using a 10% serum concentration. Cells growing in smaller serum concentrations should be brought back to 10% serum before adaptation is attempted. The following method is a basic guide to the procedure.

1. Two days prior to subculturing, nourish the cell line with defined medium containing 5% FBS and 5% alternative serum (known as "adaptation serum").
2. 48 hours afterward, trypsinize the cells and split them using "adaptation medium."
3. Nourish the cells with "adaptation medium" until confluent.
4. Repeat steps 2 and 3 for 1-2 subcultures.
5. Split the confluent cells using media containing 10% alternative serum

NOTE: Most heteroploid lines can be grown on a variety of sera without affecting the growth rate. Diploid lines are more fastidious but can be adapted. Primary cell cultures should be initiated on newborn calf serum wherever possible.

Eye Examination of Cell Cultures

Before commencing, the general health of any culture should be evaluated. This can be done quickly and quantitatively by making the following observations.

1. Check the pH of the culture by examining the color. As a culture becomes more acidic, the indicator (phenol red) shifts from red to yellow. In contrast, as a culture becomes more alkaline, its color shifts from red to fuscia. In general, cells can tolerate slight acidity better than slight basicity. Any shift above pH 7.6 is particularly detrimental. Until you become familiar with the color of the indicator at various pH levels, use color standards for comparison.
2. Cell attachment of monolayer cultures should be to the well and spread out evenly. If cells are floating in the culture, determine if they are in a state of division or if they are dying. A dying cell will have an irregular morphology (alternatively, you can test for cell viability using Trypan Blue from ICN).
3. The "percent confluency" or growth rate of a culture can be estimated by following it toward the development of a full cell sheet (i.e. a "confluent culture"). By comparing the amount of space covered by cells with the unoccupied spaces you can estimate the percent confluency.
4. The cell shape is an important guide. Round cells in an uncrowded culture are not a good indicator unless these happen to be dividing cells. Look for doublets or dividing cells. Get to know the effect of crowding on cell shape.
5. Look for "Giant Cells." The number of "giant cells" will increase as a culture ages or declines in "well-being." The frequency of "giant cells" should be relatively low and constant under uniform culture conditions.
6. One of the most valuable early indicators in assessing the success of a "culture split" is the rate at which the cells in the newly established cultures attach and spread out. Attachment within an hour or two suggests that the cells have not been traumatized and that the *in vitro* environment is not grossly abnormal. Longer attachment times are suggestive of problems. Nevertheless, good cultures may result even if attachment does not occur for four hours.
7. Learn to recognize the range of cell shapes and growth patterns exhibited by each cell line. For example, many transformed cells will "pile up" due to a lack of contact inhibition. This effect becomes more pronounced as the culture becomes overcrowded.

Subculturing Protocol - Anchorage-Dependent Established Cell Lines (Long Method)

1. Aseptically decant growth medium from the flask and replace with EBSS without calcium and magnesium (cat. no. 18-004-49) as follows:

   a. 25 cm^2 flask approximately 5 ml
   b. 75 cm^2 flask approximately 10 ml
   c. 120 cm^2 flask approximately 25 ml
2. Carefully wash the monolayer with a salt solution to remove any excess serum.
3. Aseptically decant the salt solution and replace with an equal amount of 0.05% trypsin/0.02% EDTA solution prepared as follows:

   a. 500 ml EBSS without calcium and magnesium (cat. no. 18004-49)
   b. 10 ml trypsin 1:250 (cat. no. 16-893-49)
   c. 5 ml 2% (w/v) EDTA (28-205-49)
4. Pass the trypsin/EDTA solution over the monolayer several times by gently racking the flasks and decant. DO NOT allow the trypsin/EDTA to be in contact with the monolayer longer than 30 seconds.
5. Lay the flask flat and incubate at room temperature until the cells detach. Flasks which have been trypsinized should be inspected regularly to prevent the cells from remaining in the trypsin/EDTA longer than is needed.
6. Wash the cells off the base of the flask with 10 ml of the required growth medium, taking special care with cells which may still be adhering to the sides and shoulders.
7. Aspirate the cell suspension carefully to break down any cell aggregates. Avoid excessive frothing.
8. Proceed as per experimental protocol or split cells as required for stock.
9. At all times, be aware of the possibility of cross-contamination. NEVER mix caps or reuse a pipette.

Subculturing Protocol - Anchorage-Dependent Cells (Short Method)

1. Decant supernatant fluid from the culture plate into a waste collection jar, taking care to use sterile technique.
2. Add 3 ml of cold trypsin (cat. no. 16-891-49) to the culture flask.
3. Incubate for 30 seconds (or longer if necessary). Examine at low magnification. When it appears that some of the cells have rounded up, but have yet to detach, decant the trypsin into a waste container. Continue to incubate the flask until virtually all cells have rounded up and can be readily dislodged.
4. Using a 10 ml pipette, add 10 ml of fresh MEM (cat. no. 12-102-49) to the T-flask. Dispensing the MEM as a strong stream will aid in dislodging the cells. NOTE: 10 ml is sufficient for a 1:2 split. Next, using the same pipette, drawup the cell suspension and quickly dispense a 5 ml aliquot into two 25 cm^2 T-flasks (one new, one old).
5. Incubate and monitor the flasks periodically beginning 30-45 minutes after inoculation. Rapid attachment (usually within 1 hour) is indicative that the split has been successful.
6. Continue to monitor the culture's progress.

Always employ sterile techniques throughout. The above volumes correspond to a 25 cm^2 T-flask.

Hemocytometer Counting and Cell Viability

Accurate enumeration of cell density is an important aspect of cell culture. Cell enumeration with a hemocytometer is the most widely used method, and it continues to be used in most cell culture laboratories including those equipped with electronic cell counters. The following review information should help in proceeding through the cell counting protocol. Cell populations are usually expressed as the number of cells/ml or as the number of cells/culture.

When viewed with a compound microscope with a 10X ocular and a 10X objective (total magnification 100X), one large square of the hemocytometer of 1x1 mm will fill the field. When using a Neubauer hemocytometer, each of the four large corner squares and the large center square are counted. When the cell count is low, <10 cells/square, all nine squares should be counted. Each large square measures 1x1 mm and is 0.1 mm deep. Hence, each large square has volume of 0.1 mm^3 or 0.0001 ml (10^{-4} ml). The final calculation takes into account:

1. How you wish to express your count (cells/ml or cells/flask).
2. The dilution (amount of saline, dye or media in ml which your cells have been suspended).
3. The number of squares counted. Hence, the number of cells/ml can be calculated as:

$$\frac{\text{Total Number of Cells Counted x Dilution x } 10^4}{\text{Total Number of Squares Counted}}$$

Trypan blue stains dead cells and is exuded by live cells.

Protocol

Proceed to make a 1:2 split of the culture. In order to determine the number of cells which were harvested from the flask, the culture must be centrifuged, the supernatant decanted and the pellet resuspended in 1 ml MEM. Aseptically remove 0.1 ml of cell suspension and mix while adding to a dilution tube containing 0.8 ml BSS and 0.1 ml trypan blue (0.4%). Sterility need not be maintained from this point forward. After 3-4 minutes, remove a few drops of the cell suspension with a Pasteur pipette and load both chambers after putting the hemocytometer coverslip in place. Count the total number of cells and the number of trypan blue stained (dead) cells in each of the four corner squares and the central square.

A. For total cell number in dilution tube (1 ml), it is calculated as follows:

$$\frac{\text{Total cell count x } 10^4}{\text{5 (Number of counted squares)}}$$

B. For non-viable cell number in dilution tube (1 ml), it is calculated as follows:

$$\frac{\text{Total number of stained cells) x } 10^4}{5}$$

C. For the total number of cells harvested from the original flask, it is calculated as follows:

$$\frac{\text{Total cell count x 10* x } 10^4}{5}$$

*A dilution or multiplication factor of 10 is used here because the aliquot removed for counting (0.1 ml) is one-tenth of the total cell suspension in the centrifuge tube.

D. For the number of cells/new culture after inoculation of the flask with 0.4 ml of cell suspension and addition of 5.6 ml MEM, it is calculated as follows:

$$\frac{\text{Total cell count x 4 x } 10^4}{5}$$

E. For the number of cells/ml in the new culture, use the following equation:

$$\frac{\text{Total Cell Count x 4 x } 10^4}{\text{5 x 6 ml}}$$

Cell Freezing

The freezing of cells is used to preserve seed stocks of any given culture, to guard against "genetic wandering" and contamination, and for long term storage of a culture not in regular use. ICN recommends that after obtaining a fresh stock of cells from primary sources, a proportion of the culture material be preserved and frozen as early as possible. Ideally, this should be done within 1 or 2 passages after obtaining the cell culture. A "seed" stock of culture material may be withdrawn at intervals, thereby ensuring a constant, identical source of cells for many years to come.

At ICN, we store our cell lines in glass ampoules designated "Seed Stock" and they are frozen as soon as possible after packaging. From the seed stock, further aliquots are taken, frozen and designated as "Working Stock." This "working stock" has sufficient ampoules for approximately one year. When the "working stock" has been depleted, another ampoule of seed stock is thawed and grown up to provide a new supply of working stock. Using this procedure, almost unlimited supplies of genetically similar material can be ensured. We do not recommend handling cells for more than 10 passages or 10 weeks in the laboratory. This ensures that changes will not occur in the cell line. It is essential that fresh back-up stocks always be available in deep-freeze. When freezing cells, the following recommendations insures high cell viability.

1. Check that cells are rapidly growing and in a phase of exponential growth before freezing. If working with finite,fibroblast cultures, they should NOT have been "confluent" for more than 24 hours. If working with a continuous cell line,harvest at70-80% confluency. Suspension cells should be spun and harvested while in exponential growth.
2. Trypsinize the monolayer as quickly as possible, using cold trypsin to minimize trypsin carry over. Use only minimal amounts of trypsin, ensuring that most is removed from the monolayer. This may be achieved by simply pouring off the trypsin or by centrifuging the cells and resuspending them in fresh medium.
3. Freeze the cells at a concentration between 2-5x10^6 cells/ml. For finite cultures, it is recommended that the entire contents of a flask be harvested, spun and the cell pellet resuspended in sufficient medium to produce one ampoule of material (i.e. enough to generate one flask worth of cells at passage number 15). If two ampules are produced from one flask, then this must be accounted for in the passage number, which in this case, a 1:2 split has been accomplished, so the passage number would be 16.
4. For established cultures, the yield is often large enough for several ampoules to be produced from one parent flask. In this case, the passage number should be increased by 1 to indicate that a passage or the equivalent of a subcultivation has occurred.
5. Keep the monolayer suspension cool to minimize damage from the cryoprotective reagent. Since suspension cells do not respond well to low temperatures, they should NOT be cooled. Place the ampoule in a controlled rate freezer that is set to cool at a rate of 1°C per minute. Allow the cells to freeze for approximately four hours before transferring them to a permanent position in the nitrogen freezer.
6. Frozen cells may be kept for short periods (4-10 weeks) at 70°C, but long term storage should always be in the vapor phase of liquid nitrogen or an equivalent temperature.

Frozen Culture Recovery

Thaw the frozen ampoules as quickly as possible by immersing them in a water bath at 37°C. Care should be taken if the ampoules have been in contact with liquid nitrogen since they may explode if nitrogen has leaked into them. Once the contents of the ampoules has been thawed, remove the cells with a narrow pipette or syringe and transfer them to a centrifuge tube or a culture flask. Dilute the cryoprotective agent by addition of growth medium to the cells slowly over 1-2 minutes. A 1:10 dilution should be made. Centrifugation and resuspension of the cell pellet in fresh medium will ensure that optimal conditions for subsequent cell growth exist. However, in many instances, a straight dilution is all that is required. If the cryoprotective reagent is NOT removed by centrifugation, then it is recommended that the culture be fed after 24 hours incubation.

Mycoplasma Contamination

Mycoplasma are prokaryotic cellular organisms and are the smallest cells capable of autonomous growth. They lack a rigid cell wall and are pleiomorphic. Additionally, they are sensitive to oxygen tension and osmotic shock. Many of the smaller mycoplasmas can pass through bacterial filters. The cell size ranges from 0.15 to 1.0 μm. They possess enough nucleic acid to direct the synthesis of about 750 different proteins which is probably the minimum number of proteins needed for cell life (Alberts, et al. 1980).

Most mycoplasmas are parasites, either in animals or in plants, though some are saprophytic in soil and sewage. They can be classified into two main groups dependent on their requirement for sterol (found in animal organ products such as trypsin and serum). Mycoplasma DO need sterol for growth, whereas, it is not essential for Acholeplasmataceae. A third group are the T-mycoplasmas. They require cholesterol, split urea and grow at a lower pH than "classical" mycoplasmas. They are the Ureaplasmataceae. Mycoplasma colonies have a typical "fried-egg" appearance on agar.

Mycoplasmas can cause economically important infections of the respiratory tract, mammary glands, genital tracts or synovia of cattle, sheep, goats, cats, mice, rats, pigs and poultry. There are eight or nine species that require man as the host. Plant associated mycoplasmas are usually found as insect transmitted diseases.

Surveys on the incidence of mycoplasma infection of cell cultures ranges from 5.8-16.5% (Del Guidice and Gardella, 1985). Some effects of mycoplasma infection on cell culture have been reviewed (McGarrity, et al., 1984). These infections DO NOT always result in microscopic alterations of cells or media. Many infections grow slowly and do not destroy host cells but can still alter the metabolism of the culture in subtle ways. The Acholeoplasmataceae can grow in cell-free media so that the properties of the medium are altered. Cell associated mycoplasmas change the biology of the cell so as to make results of experiments performed on the cultures in question. Signs of chronic mycoplasma infection in cell cultures include reduced rate of proliferation, reduced saturation density and agglutination during growth in suspension. Primary cell cultures have lower rates of mycoplasma infection than continuous cell lines (Barille, 1962).

The exact manner in which contamination of cell cultures occurs may be impossible to ascertain in any given instance. Studies by Barile and others (Barile, et al., 1978) have demonstrated that mycoplasma infection or contamination of cultures is the result of a number of factors, involving the investigator, the type of cell culture used and the media used in handling the cells. McGarrity (1976) concluded that mycoplasma-infected cultures are the most common source of further contamination. He listed recommendations for prevention and control of mycoplasma infection. These can be summarized as the following combination of practices:

- *The use of primary cultures rather than propagated cell types.*
- *The use of heat-inactivated serum.*
- *The avoidance of direct mouth pipetting.*
- *The strict enforcement of good aseptic techniques.*
- *The use of antibiotic-free media.*
- *The use of protective clothing by technicians.*
- *The use of premium cell biology vendors such as ICN where maximum QC measures are employed.*
- *The use of prophylactic tools such as the ICN MycoTest™ and Mycoplasma Stain Kit for mycoplasma detection.*
- *The use of Mycoplasma Removal Agent (MRA) for mycoplasma decontamination.*

Mycoplasma Isolation on Agar

The primary consideration in mycoplasma testing is the specimen. An established cell sheet grown on medium free of bacterial inhibitors is probably best. Antibiotics which may be mycoplasma-static will impair isolation attempts but fail to cure the cells. In general, antibiotics are NOT recommended for cell propagation.

Trypsinization also deters mycoplasma isolation. Infected cells tested directly after trypsin treatment may not produce mycoplasma growth on agar. After cell outgrowth, mycoplasma can then be isolated in high titer. A cell suspension should be obtained by scraping the cells from the surface of the culture vessel.

It is extremely important to culture a cell suspension rather than the supernatant fluid. Although mycoplasma is shed into the fluid, it is mainly bound to the cell surface. Also, there is a carry-over of nutrients with the cells which nourish the mycoplasma on axenic agar. This point is worth noting because colonies of fastidious mycoplasma is found to develop adjacent to deposited clumps of tissue culture.

Cell Proliferation Assay (CPA)

The Cell Proliferation Assay is used to monitor the rate of cellular growth in tissue culture systems. Quantifying the effects of hormones, growth factors, drugs, and therapeutic agents on cellular growth is a common application in today's biomedical research laboratories. Cellular growth studies are also an integral part of research in labs working on oncogenes and cell cycle regulation.

In the CPA, cellular reproduction is quantified by measuring the total amount of *de novo* cellular DNA being produced. Every time a cell divides, it replicates its DNA. As such, the total amount of *de novo* DNA in a cell culture is a proportionate indicator of the amount of cell growth that has taken place. *De novo* DNA production is most easily monitored by adding ^{3}H thymidine, a radioactive DNA precursor, to a cell culture system. Although ^{3}H thymidine is by far the most commonly used label for this application, ^{14}C thymidine and ^{32}P orthophosphate are also used. When tissue culture cells are incubated with ^{3}H thymidine, the thymidine is absorbed by the nuclei of the cells where it is then phosphorylated to TTP. This nucleotide triphosphate is then incorporated into cellular DNA at a rate that is proportionate to the cell growth rate. After labeling of the cells for a predetermined amount of time, the cells are lysed and the radioactive DNA is counted in a Liquid Scintillation Counter.

General Protocol

1. For the cell culture, the CPA has been employed with a variety of cell lines and tissues. It may be used with both primary and established cell cultures. In culturing primary cell lines, the animal is sacrificed and the tissue is removed. The tissue is treated with enzymes such as trypsin and collagenase such that the cells of interest are separated from the tissue itself. The separated cells are the cultured for 1-3 days in order to obtain a culture which is confluent. Culturing is most often performed in 96 well microplates since they allow large numbers of samples to assayed simultaneously.
2. When radiolabeling with ^{3}H thymidine, mitogenic agents such as growth factors are frequently added to the culture media immediately prior to cell labeling. ICN currently offers more than 150 growth factors, the largest selection currently available from any company. Radioactive thymidine is added to culture media such that the final concen- tration is 10-100 μCi/ml. The radioactive thymidine is taken up by the cell nuclei and converted to TTP resulting in incorporation into *de novo* DNA. Each time the cell population reproduces itself, the total amount of DNA in the cell culture doubles. ICN offers a wide range of tritiated thymidine. Please refer to the radiochemicals section for details.
3. The higher the specific activity, the more rapidly the thymidine will undergo radiolytic decay during storage. Radiolytic decay is the process whereby the radioactive particles from an isotope break chemical bonds in a molecule. In general, ^{3}H thymidine stored at 4°C undergo radiolytic decay at a rate of 1% per month.
4. The radiolabeled cells must be washed to remove unincorporated thymidine and then harvested onto filter paper for LSC counting. Manual techniques for doing this are available but they are laborious and time consuming. ICN recommends using a cell harvester for large scale automation of cell washing and harvesting. Typically, a wash solution is fed into each well in order to wash unincorporated label from the cells. Simultaneously, the well contents are aspirated onto a solid filter support, usually a glass fiber filter. The net effect is that the cells and cell debris from each well are deposited on a filter mat. More than 98% of the particulate material is retained, whereas, the unincorporated radioactivity is washed away.
5. The most convenient and most rapid method for quantifying ^{3}H thymidine filter samples is with a liquid scintillation counter. ICN recommends the use of CytoScint™ ES, specially formulated for maximum ^{3}H counting efficiency, when working with filters of all types. This allows for most samples collected on solid supports to be counted in one easy step without pre-treatment requirements. The presence of small amounts of water will actually enhance the solubilization of most samples in CytoScint™ ES.

2-Deoxyglucose Uptake - ^{3}H and ^{14}C

This assay can be made using cells growing on any size or type of dish. As a rough guide, cells that are growing on a 35 mm dish will generate a sufficiently large signal using 2 μCi of label. We recommend performing this procedure in a 37°C walk-in incubator. First, the dishes are washed with ICN CELLect™ PBS that has been warmed to 37°C. Then add 1 ml of PBS with 1% ICN CELLect™ calf serum and 2 mCi of 2-deoxy-D-glucose ^{3}H. let the cells incubate in this solution for 10 minutes at 37°C exposed to the air.

Note: If you are studying the effect of growth factors on cells, we recommend NOT adding any serum to the PBS. Following the labeling step, aspirate the label and wash the cells four cycles with PBS. If a Lowry is going to be performed in the cells, rinse with serum-free PBS. If all the dishes contain the same number of cells and none have detached during the procedure, you can dissolve the cells in 1 ml of 1% SDS and count them directly in 10 ml of ICN Ecolite™ liquid scintillation fluid. Alternatively, if there is significant variation in cell number and/or the cells have become detached during washing, it is necessary to adjust the volume of SDS to correct for the number of cells being counted. Glucose transport rates should be normalized to cell protein rather than cell number, because larger cells can be expected to transport more glucose than smaller cells. Normalization is effected by dissolving the labeled cells in Lowry solution and counting 50% of this. The total protein is measured in the other 50% of solution.

^{35}S Methionine and Cysteine Labeling of Cells

Efficient labeling of mammalian cell proteins generally requires the use of deficient media. For customers labeling with ^{35}S methionine, we recommend the use of RPMI, DMEM, and MEM deficient in methionine. When labeling with Tran^{35}S-label™, a mixture of both ^{35}S methionine and ^{35}S cysteine, the use of methionine deficient media is sufficient. However, for optimal incorporation, particularly with proteins containing several cysteine residues, the use of methionine/cysteine deficient media is recommended.

Once you have prepared the deficient media, it should contain no more than 10% of the normal concentration of methionine or cysteine. The exact amount of supplemental methionine added will depend on the particular cell line being labeled. For long term labeling with Tran^{35}S-label™, a small amount of supplemental cysteine should be added. At around 8 hours after beginning the labeling reaction, the cells will have depleted their intracellular stores of cysteine and the amount of ^{35}S cysteine may not be enough to sustain continued growth.

Labeling should be performed using 10% serum and half the usual amount of medium. Some cell lines, particularly transformed ones such as rous transformed chicken cells, may require supplemental methionine, as high as 25% of normal. In general, we recommend beginning with deficient media devoid of methionine and adding supplemental methionine if it appears to be necessary.

The ideal length of time for labeling cells with ^{35}S methionine and ^{35}S cysteine depends on the protein of interest. When labeling unstable proteins, a short label of no more than 2 hours is best. With stable proteins, a longer label may be preferable. The key factor is the biological half-life of the protein of interest versus that of the other cellular proteins which will form background bands on the subsequent gel. In general, the biological half-life for total cellular proteins is 45-50 hours.

^{32}P Orthophosphate Labeling of Cells

As in methionine labeling, the use of deficient media is strongly recommended in order to obtain maximal incorporation. ICN recommends using only half the amount of media as in normal labeling. Additionally, the use of serum which has been dialyzed against phosphate-free saline will help increase incorporation.

Labeling time with ^{32}P orthophosphate is somewhat different than with ^{35}S methionine. Generally, the phosphates in proteins undergo continual turnover. As such, both old and newly synthesized proteins incorporate label soon after it has been added to the cells. We recommend a short labeling period with ^{32}P orthophosphate since the labeling of RNA and DNA is less pronounced than in cells labeled overnight.

In contrast, when studying steady-state abundance of phosphates in proteins, lipids or RNA, an overnight labeling is optimal. After long-term labeling, the specific activity of the ATP pool in the cell and the phosphates in the macromolecules will have equilibrated with that in the medium. Additionally, the amount of ^{32}P orthophosphate found in the proteins, lipids and RNA should now reflect the total amount of phosphate present rather than the turnover rate of the phosphate.

Chromium Release Assay

In cellular immunology, the mechanisms in which the body wards off foreign substances such as viruses, bacteria and toxic agents is a major area of investigation. the key player in the body's defense system against these toxic invaders is the cytotoxic T-lymphocyte. These lymphocytes have receptors which recognize antigens present on the surface of foreign cells. Recognition of a foreign antigen by the receptor allows the cytotoxic T-lymphocyte to proceed to kill the invading cells.

In vitro methods, known as Chromium Release Assays, have been developed which allow researchers to quantify this cytotoxic phenomenon. Among other things, these assays allow one to determine the number of lymphocytes being produced in response to an infection or a drug treatment and how effectively these cells kill the foreign antigen bearing cells.

The basis of the assay is chromium 51 which has the property of binding to the cellular proteins of cultured cells. Target cells are pre-labeled by incubation with ^{51}Cr. These target cells are then incubated with effector cells and the amount of radioactivity which is released in the supernatant is taken as an indicator of the amount of lysis which has occurred. This assay takes advantage of the fact that cytotoxic T-cells kill their target cells by disrupting the integrity of the cell membrane thereby allowing the release of ^{51}Cr bound to protein from the target cell. A simple calculation of the amount of cell bound ^{51}Cr versus free ^{51}Cr allows one to quantify the amount of cellular cytotoxicity.

Procedure

1. Cultured cells are given a dose of radiolabeled sodium chromate ^{51}Cr. As the cells grow, they uptake the ^{51}Cr into cellular proteins. At the end of the incubation, the cells are washed to remove any unincorporated label.
2. Effector (E) cells are incubated with chromium labeled target (T) cells at various cell-ratios (denoted as E/T ratios). Incubation is typically performed in a multiwell plate for a period of 2-4 hours at 37°C. Cytotoxic effects mediated by the effector cells are quantitated by measuring the released ^{51}Cr-protein complex. After incubation, the multiwell plate is centrifuged to pellet the cells to the bottom of the plate. An aliquot isremoved from each of the wells and quantitated using a gamma counter. The rest of the well's contents (cells and supernatant) is removed using one of several commercially available cell harvesters which then transfers the material to glass fiber filters. Each of the filters is counted in a gamma counter.
3. The percent cytoxicity/cell lysis is calculated based on the total amount of ^{51}Cr released. In short, the calculation is as follows:

Percent Specific Lysis =

$$\frac{\text{^{51}Cr Released by T - ^{51}Cr Released by Normal Cells}}{\text{Maximum ^{51}Cr Released - ^{51}Cr Released by Normal Cells}}$$

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Basal Medium Eagle (BME) (Modified)

Basal Medium Eagle (BME), originally developed by Harry Eagle and the predecessor of MEM and DMEM, is one of the most commonly used of all cell culture media. According to the Tissue Culture Association (TCA), the name "Eagle's Basal Medium" refers only to the several formulations developed by Eagle to support HeLa cells. This medium is the result of numerous studies from the late 1950's analyzing the nutrient requirements and other components essential for the growth of cells in culture. Traditionally, BME has been used in studies to measure the growth response of normal (WI-38) and transformed (HeLa) cells in monolayer culture. BME may be used for a broad variety of cell lines when supplemented with serum.

COMPONENT	**1200254 1X Liquid** mg/L	**1200354 1X Liquid** mg/L	**1200454 1X Liquid** mg/L	**1200654 1X Liquid** mg/L	**1203254 1X Liquid** mg/L	**1203354 1X Liquid** mg/L	**1400054 10X Liquid** mg/L	1000122 Powder mg/L	**1003122 Powder** mg/L	**1094122 Diploid Powder** mg/L	1100022 Auto-Pow™ mg/L
AMINO ACIDS											
L-Arginine • HCl	21.06	21.06	21.06	21.06	21.06	21.06	210.60	21.06	21.06	21.06	21.06
L-Cystine • HCl	15.11	15.11	15.11	15.11	15.11	15.11	151.10	15.11	15.11	15.11	15.11
L-Glutamine	--	292.30	--	--	--	292.30	--	292.30	292.30	292.30	--
L-Histidine • HCl • H_2O	10.50	10.50	10.50	10.50	10.50	10.50	105.00	10.50	10.50	10.50	10.50
L-Isoleucine	26.23	26.23	26.23	26.23	26.23	26.23	262.30	26.23	26.23	26.23	26.23
L-Leucine	26.23	26.23	26.23	26.23	26.23	26.23	262.30	26.23	26.23	26.23	26.23
L-Lysine • HCl	36.53	36.53	36.53	36.53	36.53	36.53	365.30	36.53	36.53	36.53	36.53
L-Methionine	7.46	7.46	7.46	7.46	7.46	7.46	74.60	7.46	7.46	7.46	7.46
L-Phenylalanine	16.51	16.51	16.51	16.51	16.51	16.51	165.10	16.51	16.51	16.51	16.51
L-Threonine	23.82	23.82	23.82	23.82	23.82	23.82	238.20	23.82	23.82	23.82	23.82
L-Tryptophan	4.08	4.08	4.08	4.08	4.08	4.08	40.80	4.08	4.08	4.08	4.08
L-Tyrosine • 2Na • $2H_2O$	25.95	25.95	25.95	25.95	25.95	25.95	259.50	25.95	25.95	25.95	25.95
L-Valine	23.43	23.43	23.43	23.43	23.43	23.43	234.30	23.43	23.43	23.43	23.43
VITAMINS											
D-Biotin	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00	2.00	2.00	2.00	20.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Pyridoxal • HCl	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
Riboflavin	0.10	0.10	0.10	0.10	0.10	0.10	1.00	0.10	0.10	0.10	0.10
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	10.00	1.00	1.00	1.00	1.00
INORGANIC SALTS											
Calcium Chloride • $2H_2O$	264.9	264.9	264.9	264.9	264.9	264.9	2649.00	264.90	185.50	264.90	264.90
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	97.70	97.70	97.70	977.00	97.70	97.70	--	97.70
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	4000.00	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic	--	--	--	--	--	--	--	--	60.00	--	--
Sodium Bicarbonate	1680.00	1680.00	--	850.00	350.00	350.00	--	--	--	--	--
Sodium Chloride	6800.00	6800.00	6800.00	6800.00	350.00	350.00	68000.00	6800.00	8000.00	6800.00	6800.00
Sodium Phosphate Monobasic (anhydrous)	140.00	140.00	140.00	140.00	140.00	140.00	1400.00	140.00	--	140.00	140.00
Sodium Phosphate Dibasic	--	--	--	--	--	--	--	--	47.50	--	--
OTHER											
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	10000.00	1000.00	1000.00	1000.00	1000.00
Phenol Red (sodium)	10.00	10.00	10.00	10.00	10.00	10.00	170.00	17.00	17.00	17.00	17.00
HEPES	--	--	4766.40	--	--	--	--	--	--	--	--
Sodium Succinate • $6H_2O$	--	--	--	--	--	--	--	--	--	--	100.00
Succinic Acid	--	--	--	--	--	--	--	--	--	--	75.00

Biggers' Medium BGJ (Modified)

This medium was orginally developed by Biggers, Gwatkin and Judah during the early 1960's while at the Wistar Institute. It is recommended for supporting the culture of adult and embryonic mammalian skeletal and cartilaginous tissue. Another modification developed by Sylvia Fitton-Jackson is further enriched over the original formula. The added amino acids and vitamins, along with the increased buffering capacity resulting from the additional phosphates, creates conditions permitting both calcification and the growth of cartilaginous embryonic bone.

COMPONENT	1297954 1X Liquid mg/L
AMINO ACIDS	
L-Arginine HCl	75.00
L-Cystine HCl	80.77
L-Glutamine	--
L-Histidine • HCl • H_2O	150.00
L-Isoleucine	30.00
L-Leucine	50.00
L-Lysine HCl	240.00
L-Methionine	50.00
L-Phenylalanine	50.00
L-Threonine	75.00
L-Tryptophan	40.00
L-Tyrosine 2Na $2H_2O$	57.66
L-Valine	65.00
VITAMINS	
D-Biotin	0.20
Choline Chloride	50.00
Folic Acid	0.20
myo-Inositol	1.00
Nicotinic Acid	20.00
D-Pantothenic Acid (hemicalcium salt)	0.50
PABA (p-Aminobenzoic Acid)	2.00
Pyridoxal-5-phosphate	0.215
Riboflavin	0.20
Thiamine HCl	2.00
DL-α-Tocopherol Phosphate • $2H_2O$	1.065
Vitamin B-12	0.04
INORGANIC SALTS	
Magnesium Sulfate • $7H_2O$	200.00
Potassium Chloride	350.00
Potassium Phosphate Monobasic	35.00
Sodium Acetate	50.00
Sodium Bicarbonate	2200.00
Sodium Chloride	6200.00
Sodium Phosphate Dibasic	140.00
OTHER	
Lactic Acid (hemicalcium salt)	522.60
D-Glucose	5000.00
Phenol Red (sodium)	20.00

CMRL 1066 Medium (Modified)

CMRL 1066, formulated in the 1950's at the Connaught Medical Research Laboratories, is a protein-free, chemically defined medium orginally developed for serum-free culture. It is beneficial for the cloning of monkey kidney cells and the continuous cultivation of sublines of L-cells. It is a less complex and extensively modified version of Medium 199, and it can be supplemented with serum to support the culture of many different cell types.

COMPONENT	1466054 10X Liquid mg/L	1066122 Powder mg/L
AMINO ACIDS		
L-Alanine	250.00	25.00
L-Arginine • HCl	700.00	70.00
L-Aspartic Acid	300.00	30.00
L-Cysteine • HCl	2333.00	233.30
L-Cystine • 2Na • H_2O	251.60	25.16
L-Glutamic Acid	750.00	75.00
L-Glutamine	--	100.00
Glycine	500.00	50.00
L-Histidine • HCl • H_2O	200.00	20.00
trans-4-Hydroxy-L-Proline	100.00	10.00
L-Isoleucine	200.00	20.00
L-Leucine	600.00	60.00
L-Lysine • HCl	700.00	70.00
L-Methionine	150.00	15.00
L-Phenylalanine	250.00	25.00
L-Proline	400.00	40.00
L-Serine	250.00	25.00
L-Threonine	300.00	30.00
L-Tryptophan	100.00	10.00
L-Tyrosine • 2Na • H_2O	576.50	57.65
L-Valine	250.00	25.00
VITAMINS		
p-Aminobutyric Acid	0.50	0.05
L-Ascorbic Acid • Na	500.00	50.00
D-Biotin	0.10	0.01
Choline Chloride	5.00	0.50
Folic Acid	0.10	0.01
myo-Inositol	0.50	0.05
Niacinamide	0.25	0.025
Nicotinic Acid	0.25	0.025
D-Pantothenic Acid (hemicalcium salt)	0.10	0.01
Pyridoxal • HCl	0.25	0.025
Pyridoxine • HCl	0.25	0.025
Riboflavin	0.10	0.01
Thiamine • HCl	0.10	0.01
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	2649.00	264.90
Magnesium Sulfate • $7H_2O$	2000.00	200.00
Potassium Chloride	4000.00	400.00
Sodium Acetate	500.00	50.00
Sodium Bicarbonate	--	--
Sodium Chloride	68000.00	6800.00
Sodium Phosphate Monobasic (anhydrous)	1400.00	140.00
OTHER		
Cholesterol	2.00	0.20
Cocarboxylase	10.00	1.00
Coenzyme A • 3Li	23.90	2.39
2′-Deoxyadenosine	100.00	10.00
2′-Deoxycytidine • HCl	100.00	10.00
2′-Deoxyguanosine	100.00	10.00
Flavin Adenine Dinucleotide • 2Na	10.56	1.056
D-Glucose	10000.00	1000.00
D-Glucuronic Acid • Na	42.00	4.20
Glutathione	100.00	10.00
5-Methyldeoxycytidine	1.00	0.10
β-NAD	71.90	7.19
β-NADP • 2Na • $2H_2O$	10.27	1.027
Phenol Red (sodium)	100.00	10.00
Thymidine	100.00	10.00
Tween 80	50.00	5.00
Uridine-5′-triphosphate • 3Na	10.00	1.00

Dulbecco's Modified Eagle's Medium (DMEM) (Modified)

Also known as DME, Dulbecco's Modified Eagle's Medium is the most widely used of modification of Eagle's Basal Medium (BME). DMEM contains four times greater concentration of amino acids, vitamins and supplementary components. The original formulation calls for 1000 mg/L of glucose, which was first employed to support the polyoma virus in primary and secondary embryonic mouse cultures. For optimal culturing of other cell types, a modification of 4500 mg/L glucose is available from ICN.

COMPONENT	1233154 1X Liquid mg/L	1233254 1X Liquid mg/L	1233354 1X Liquid mg/L	1233454 1X Liquid mg/L	1233654 1X Liquid mg/L	1233854 1X Liquid mg/L	1234254 1X Liquid mg/L	1642154 1X Liquid mg/L	1642254 1X Liquid mg/L	1642354 1X Liquid mg/L
AMINO ACIDS										
L-Arginine • HCl	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00	84.00
L-Cystine • 2HCl	62.57	62.57	62.57	62.57	62.57	62.57	62.57	62.57	32.57	62.57
L-Glutamine	584.00	--	584.00	--	584.00	--	--	--	--	--
Glycine	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
L-Histidine • HCl • H_2O	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
L-Isoleucine	104.80	104.80	104.80	104.80	104.80	104.80	104.80	104.80	104.80	104.80
L-Leucine	104.80	104.80	104.80	104.80	104.80	104.80	104.80	--	104.80	104.80
L-Lysine • HCl	146.20	146.20	146.20	146.20	146.20	146.20	146.20	146.20	146.20	146.20
L-Methionine	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	--	--
L-Phenylalanine	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00	66.00
L-Serine	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00	42.00
L-Threonine	95.20	95.20	95.20	95.20	95.20	95.20	95.20	95.20	95.20	95.20
L-Tryptophan	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00	16.00
L-Tyrosine • 2Na • $2H_2O$	103.80	103.80	103.80	103.80	103.80	103.80	103.80	103.80	103.80	103.80
L-Valine	94.00	94.00	94.00	94.00	94.00	94.00	94.00	94.00	94.00	94.00
VITAMINS										
Choline Chloride	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
myo-Inositol	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20	7.20
Niacinamide	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
D-Pantothenic Acid (hemicalcium salt)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Pyridoxine • HCl	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Riboflavin	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Thiamine • HCl	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
INORGANIC SALTS										
Calcium Chloride (anhydrous)	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
Ferric Nitrate • $9H_2O$	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	97.70	97.70	97.70	97.70	97.70	97.70	97.70
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00
Sodium Bicarbonate	3700.00	3700.00	3700.00	--	3700.00	3700.00	3700.00	3700.00	3700.00	3700.00
Sodium Chloride	6400.00	6400.00	6400.00	6400.00	6400.00	6400.00	6400.00	6400.00	6400.00	6400.00
Sodium Phosphate Monobasic (anhydrous)	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	125.00	--
OTHER										
D-Glucose	4500.00	4500.00	4500.00	4500.00	4500.00	4500.00	1000.00	4500.00	4500.00	4500.00
HEPES	5958.00	--	--	4766.00	--	--	--	--	--	--
Phenol Red (sodium)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Pyruvic Acid • Na	110.00	110.00	110.00	110.00	--	--	110.00	110.00	110.00	110.00

Dulbecco's Modified Eagle's Medium (DMEM) (Modified)

Also known as DME, Dulbecco's Modified Eagle's Medium is the most widely used of modification of Eagle's Basal Medium (BME). DMEM contains four times greater concentration of amino acids, vitamins and supplementary components. The original formulation calls for 1000 mg/L of glucose, which was first employed to support the polyoma virus in primary and secondary embryonic mouse cultures. For optimal culturing of other cell types, a modification of 4500 mg/L glucose is available from ICN.

COMPONENT	1642454 1X Liquid mg/L	1642754 1X Liquid mg/L	1642954 1X Liquid mg/L	1433054 10X Liquid mg/L	1033122 Powder mg/L	1033222 Powder mg/L	1033522 Powder mg/L
AMINO ACIDS							
L-Arginine • HCl	84.00	84.00	84.00	840.00	84.00	84.00	84.00
L-Cystine • HCl	--	62.57	62.57	625.70	62.57	62.57	62.57
L-Glutamine	--	--	--	--	584.00	584.00	584.00
Glycine	30.00	30.00	30.00	300.00	30.00	30.00	30.00
L-Histidine • HCl • H_2O	42.00	42.00	42.00	420.00	42.00	42.00	42.00
L-Isoleucine	104.80	104.80	104.80	1048.00	104.80	104.80	104.80
L-Leucine	104.80	104.80	104.80	1048.00	104.80	104.80	104.80
L-Lysine • HCl	146.20	146.20	146.20	1462.00	146.20	146.20	146.20
L-Methionine	--	30.00	30.00	300.00	30.00	30.00	30.00
L-Phenylalanine	66.00	66.00	66.00	660.00	66.00	66.00	66.00
L-Serine	42.00	42.00	42.00	420.00	42.00	42.00	42.00
L-Threonine	95.20	95.20	95.20	952.00	95.20	95.20	95.20
L-Tryptophan	16.00	16.00	16.00	160.00	16.00	16.00	16.00
L-Tyrosine • 2Na • $2H_2O$	103.80	103.80	103.80	1038.00	103.80	103.80	103.80
L-Valine	94.00	94.00	94.00	940.00	94.00	94.00	94.00
VITAMINS							
Choline Chloride	4.00	4.00	4.00	40.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	40.00	4.00	4.00	4.00
myo-Inositol	7.20	7.20	--	72.00	7.20	7.20	7.20
Niacinamide	4.00	4.00	4.00	40.00	4.00	4.00	4.00
D-Pantothenic Acid (hemicalcium salt)	4.00	4.00	4.00	40.00	4.00	4.00	4.00
Pyridoxine • HCl	4.00	4.00	4.00	40.00	4.00	4.00	4.00
Riboflavin	0.40	0.40	0.40	4.00	0.40	0.40	0.40
Thiamine • HCl	4.00	4.00	4.00	40.00	4.00	4.00	4.00
INORGANIC SALTS							
Calcium Chloride • $2H_2O$	200.00	200.00	200.00	2000.00	200.00	200.00	200.00
Ferric Nitrate • $9H_2O$	0.10	0.10	0.10	1.00	0.10	0.10	0.10
Magnesium Sulfate	97.70	97.70	97.70	977.00	97.70	97.70	97.70
Potassium Chloride	400.00	400.00	400.00	4000.00	400.00	400.00	400.00
Sodium Bicarbonate	3700.00	3700.00	3700.00	--	--	--	--
Sodium Chloride	6400.00	6400.00	6400.00	64000.00	6400.00	6400.00	6400.00
Sodium Phosphate Monobasic (anhydrous)	125.00	125.00	125.00	1250.00	125.00	125.00	125.00
OTHER							
D-Glucose	4500.00	4500.00	4500.00	45000.00	4500.00	4500.00	4500.00
HEPES	--	--	--	--	--	--	4766.00
Phenol Red (sodium)	15.00	--	15.00	150.00	15.00	15.00	15.00
Pyruvic Acid • Na	110.00	110.00	110.00	1100.00	110.00	--	110.00

DMEM/F-12 Nutrient Mixture (Modified)

For over a decade, the interest in serum-free cell culture has steadily grown. This medium is a 1:1 mixture of DMEM and Ham's F-12 and specially suited for culturing cells without serum supplementation. It is one of the most universal media for serum-free cell culture technigques. HEPES buffer is included in the formulation at 15 mM for added buffering capacity which is lost due to the elimination of serum. Please see the Serum-Free Cell Culture section for information on ICN's TCH™, TCM™ and TM-235™ serum replacement products.

COMPONENT	1246754 1X Liquid mg/L	1046822 Powder mg/L	1046922 Powder mg/L
AMINO ACIDS			
L-Alanine	4.455	4.455	4.455
L-Arginine • HCl	147.40	147.40	147.40
L-Asparagine • H_2O	7.505	7.505	7.505
L-Aspartic Acid	6.655	6.655	6.655
L-Cysteine • HCl • H_2O	17.56	17.56	17.56
L-Cystine • 2HCl	31.29	31.29	31.29
L-Glutamic Acid	7.355	7.355	7.355
L-Glutamine	--	--	--
Glycine	18.75	18.75	18.75
L-Histidine • HCl • H_2O	31.48	31.48	31.48
L-Isoleucine	54.37	54.37	54.37
L-Leucine	58.95	58.95	58.95
L-Lysine • HCl	91.35	91.35	91.35
L-Methionine	17.24	17.24	17.24
L-Phenylalanine	35.48	35.48	35.48
L-Proline	17.25	17.25	17.25
L-Serine	26.25	26.25	26.25
L-Threonine	53.55	53.55	53.55
L-Tryptophan	9.02	9.02	9.02
L-Tyrosine • 2Na • $2H_2O$	55.81	55.81	55.81
L-Valine	52.85	52.85	52.85
VITAMINS			
D-Biotin	0.00365	0.00365	0.00365
Choline Chloride	8.98	8.98	8.98
Folic Acid	2.65	2.65	2.65
myo-Inositol	12.61	12.61	12.61
Niacinamide	2.019	2.019	2.019
D-Pantothenic Acid (hemicalcium salt)	2.24	2.24	2.24
Pyridoxine • HCl	2.031	2.031	2.031
Riboflavin	0.219	0.219	0.219
Thiamine • HCl	2.17	2.17	2.17
Vitamin B-12	0.68	0.68	0.68
INORGANIC SALTS			
Calcium Chloride (anhydrous)	116.65	116.65	116.65
Cupric Sulfate (anhydrous)	0.0008	0.0008	0.0008
Ferric Nitrate • $9H_2O$	0.05	0.05	0.05
Ferrous Sulfate • 7H2O	0.4170	0.4170	0.4170
Magnesium Sulfate (anhydrous)	84.95	84.95	84.95
Potassium Chloride	311.80	311.80	311.80
Sodium Bicarbonate	2438.00	--	--
Sodium Chloride	7000.00	7000.00	7000.00
Sodium Phosphate Monobasic • H_2O	62.50	62.50	62.50
Sodium Phosphate Dibasic (anhydrous)	71.00	71.00	71.00
Zinc Sulfate • 7H2O	0.4315	0.4315	0.4315
OTHER			
D-Glucose	3151.00	3151.00	3151.00
Hypoxanthine • Na	2.385	2.385	2.385
Methyl Linoleate	0.044	0.044	0.044
Phenol Red (sodium)	8.10	8.10	8.10
Putrescine • 2HCl	0.08055	0.08055	0.08055
Pyruvic Acid • Na	110.00	110.00	--
DL-Thioctic Acid	0.105	0.105	0.105
Thymidine	0.36	0.36	0.36

Fischer's Medium (Modified)

This medium was originally formulated for the support of serially propagated cells from leukemic mice. Initial studies, conducted using animals, were examined for chemotherapeutic resistance. Fischer's medium allowed for parallel *in vitro* studies. It will support the clonal reproduction of cells, especially lymphoblasts derived from prmary explants or from cultured cells.

COMPONENT	1282254 1X Liquid mg/L	1082122 Powder mg/L
AMINO ACIDS		
L-Arginine • HCl	15.00	15.00
L-Asparagine • H_2O	11.36	11.36
L-Cystine • 2Na • H_2O	25.16	25.16
L-Glutamine	--	200.00
L-Histidine • HCl • H_2O	81.07	81.07
L-Isoleucine	75.00	75.00
L-Leucine	30.00	30.00
L-Lysine • HCl	50.00	50.00
L-Methionine	100.00	100.00
L-Phenylalanine	60.00	60.00
L-Serine	15.00	15.00
L-Threonine	40.00	40.00
L-Tryptophan	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	86.48	86.48
L-Valine	70.00	70.00
VITAMINS		
D-Biotin	0.01	0.01
Choline Chloride	1.50	1.50
Folic Acid	10.00	10.00
myo-Inositol	1.50	1.50
Niacinamide	0.50	0.50
D-Pantothenic Acid (hemicalcium salt)	0.50	0.50
Pyridoxal • HCl	0.50	0.50
Riboflavin	0.50	0.50
Thiamine • HCl	1.00	1.00
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	91.00	91.00
Magnesium Sulfate • $7H_2O$	121.20	121.20
Potassium Chloride	400.00	400.00
Sodium Bicarbonate	1125.00	--
Sodium Chloride	8000.00	8000.00
Sodium Phosphate Monobasic • H_2O	69.00	69.00
Sodium Phosphate Dibasic (anhydrous)	60.00	60.00
OTHER		
D-Glucose	1000.00	1000.00
Phenol Red (sodium)	5.00	5.00

Glasgow Minimum Essential Medium (GMEM) (Modified)

GMEM (BHK-21 medium) is another modification of Eagle's Basal Medium developed by Ian MacPherson and Michael Stoker. It is formulated for examining the genetic factors affecting cell competence. The polyoma virus was used to transform fibroblast clones (BHK-21 clone 13) from baby hamster kidney cell culture. In this method, Eagle's medium was modified with 10% serum, 10% tryptose phosphate broth and double the concentration of amino acids and vitamins.

COMPONENT	1230254 1X Liquid mg/L	1230354 1X Liquid mg/L	1430054 10X Liquid mg/L	1030122 Powder mg/L
AMINO ACIDS				
L-Arginine • HCl	42.12	42.12	421.20	42.12
L-Cystine • 2Na • H_2O	30.22	30.22	302.20	30.22
L-Glutamine	--	584.60	--	584.60
L-Histidine • HCl • H_2O	21.00	21.00	210.00	21.00
L-Isoleucine	52.46	52.46	524.60	52.46
L-Leucine	52.46	52.46	524.60	52.46
L-Lysine • HCl	73.06	73.06	730.60	73.06
L-Methionine	14.92	14.92	149.20	14.92
L-Phenylalanine	33.02	33.02	330.20	33.02
L-Threonine	47.64	47.64	476.40	47.64
L-Tryptophan	8.16	8.16	81.60	8.16
L-Tyrosine • 2Na • $2H_2O$	51.90	51.90	519.00	51.90
L-Valine	46.86	46.86	468.60	46.86
VITAMINS				
Choline Chloride	2.00	2.00	20.00	--
Folic Acid	2.00	2.00	20.00	2.00
myo-Inositol	4.00	4.00	40.00	4.00
Niacinamide	2.00	2.00	20.00	2.00
D-Pantothenic Acid (hemicalcium salt)	2.00	2.00	20.00	2.00
Pyridoxal • HCl	2.00	2.00	20.00	2.00
Riboflavin	0.20	0.20	2.00	0.20
Thiamine • HCl	2.00	2.00	20.00	2.00
INORGANIC SALTS				
Calcium Chloride • $2H_2O$	264.90	264.90	2649.00	264.90
Ferric Nitrate • $9H_2O$	0.10	0.10	1.00	0.10
Magnesium Sulfate • $7H_2O$	200.00	200.00	2000.00	200.00
Potassium Chloride	400.00	400.00	4000.00	400.00
Sodium Bicarbonate	2750.00	2750.00	--	--
Sodium Chloride	6400.00	6400.00	64000.00	6400.00
Sodium Phosphate Monobasic • H_2O	123.80	123.80	1238.00	123.80
OTHER				
Choline Bitartrate	--	--	--	3.60
D-Glucose	4500.00	4500.00	45000.00	4500.00
Phenol Red (sodium)	15.00	15.00	150.00	15.00
Sodium Succinate • $6H_2O$	--	--	--	100.00
Succinic Acid	--	--	--	75.00

Grace's Insect Medium (Modified)

Grace's Medium was originally developed to support Australian emperor gum moth cells. It is a modification of Wyatt's medium resembling the chemical composition of hemolymph from *Bombyx mori*. Grace established the first continuous cell lines using this medium. Prior to use, the medium is typically supplemented with serum, yeast extract, lactalbumin hydrolysate and albumin (BSA) in varying amounts and combinations. Grace's is used to culture dipteran and lepidopteran cell ines and a variety of other insect cell types. The Baculovirus Protein Expression System recommends the use of Grace's medium.

COMPONENT	2700054 1X Liquid mg/L	2710154 Hink's TNM-FH 1X Liquid mg/L	1127122 Hink's TNM-FH Powder mg/L
AMINO ACIDS			
β-Alanine	200.00	200.00	200.00
L-Alanine	225.00	225.00	225.00
L-Arginine • HCl	700.00	700.00	700.00
L-Aspartic Acid	350.00	350.00	350.00
L-Asparagine • H_2O	397.70	397.70	397.70
L-Cystine • 2Na • H_2O	24.13	24.13	24.13
L-Glutamic Acid	600.00	600.00	600.00
L-Glutamine	600.00	600.00	600.00
Glycine	650.00	650.00	650.00
L-Histidine • HCl • H_2O	3378.00	3378.00	3378.00
L-Isoleucine	50.00	50.00	50.00
L-Leucine	75.00	75.00	75.00
L-Lysine • HCl	625.00	625.00	625.00
L-Methionine	50.00	50.00	50.00
L-Phenylalanine	150.00	150.00	150.00
L-Proline	350.00	350.00	350.00
DL-Serine	1100.00	1100.00	1100.00
L-Threonine	175.00	175.00	175.00
L-Tryptophan	100.00	100.00	100.00
L-Tyrosine • 2Na • $2H_2O$	72.01	72.01	72.01
L-Valine	100.00	100.00	100.00
VITAMINS			
p-Aminobenzoic Acid	0.02	0.02	0.02
D-Biotin	0.01	0.01	0.01
Choline Chloride	0.20	0.20	0.20
Folic Acid	0.02	0.02	0.02
myo-Inositol	0.02	0.02	0.02
Nicotinic Acid	0.02	0.02	0.02
D-Pantothenic Acid (hemicalcium salt)	0.02	0.02	0.02
Pyridoxine • HCl	0.02	0.02	0.02
Riboflavin	0.02	0.02	0.02
Thiamine • HCl	0.02	0.02	0.02
INORGANIC SALTS			
Calcium Chloride • $2H_2O$	1325.00	1325.00	1325.00
Magnesium Chloride • $6H_2O$	2280.00	2280.00	2280.00
Magnesium Sulfate • $7H_2O$	2780.00	2780.00	2780.00
Potassium Chloride	2240.00	2240.00	2240.00
Sodium Bicarbonate	350.00	350.00	350.00
Sodium Phosphate Monobasic • H_2O	1008.00	1008.00	1008.00
Succinic Acid (free acid)	60.00	60.00	60.00
OTHER			
D(-)-Fructose	400.00	400.00	400.00
Fumaric Acid (free acid)	55.00	55.00	55.00
D-Glucose	700.00	700.00	700.00
α-Ketoglutaric Acid	370.00	370.00	370.00
	--	3333.30	3333.30
L(-)-Malic Acid (free acid)	670.00	670.00	670.00
Sucrose	26680.00	26680.00	26680.00
Yeast Hydrolysate	--	3333.30	3333.30

Ham's F-10 Nutrient Medium (Modified)

Originally, Ham's Nutrient Mixtures were developed for the clonal growth of several Chinese hamster ovary (CHO) clone cells, Hela cells and mouse L-cells. Both F-10 and F-12 are formulated for use with or without serum, depending on the type of cells being cultured. F-10 has demonstrated satisfactory growth of human diploid cells, white blood cells for chromosomal analysis and primary rat, rabbit and chicken tissue explants. Ham's F-12 is well suited for the growth of primary rat hepatocytes and prostate epithelial cells. It is also the medium of choice for toxicity assays using CHO cells. ICN also offers Ham's F-12 supplemented with 25 mM HEPES for improved buffering in the optimum pH range of 7.2-7.4.

COMPONENT	1240254 1X Liquid mg/L	1240354 1X Liquid mg/L	1240454 1X Liquid mg/L	1440054 10X Liquid mg/L	1040222 Powder mg/L	1040122 Powder mg/L
AMINO ACIDS						
L-Alanine	9.00	9.00	9.00	90.00	9.00	9.00
L-Arginine • HCl	211.00	211.00	211.00	2110.00	211.00	211.00
L-Asparagine • H_2O	15.00	15.00	15.00	150.00	15.00	15.00
L-Aspartic Acid	13.30	13.30	13.30	133.00	13.30	13.30
L-Cysteine • HCl	31.50	31.50	31.50	315.00	31.50	31.50
L-Glutamic Acid	14.70	14.70	14.70	147.00	14.70	14.70
L-Glutamine	--	146.20	--	--	146.20	146.20
Glycine	7.50	7.50	7.50	75.00	7.50	7.50
L-Histidine • HCl • H_2O	23.00	23.00	23.00	230.00	23.00	23.00
L-Isoleucine	2.60	2.60	2.60	26.00	2.60	2.60
L-Leucine	13.00	13.00	13.00	130.00	13.00	13.00
L-Lysine • HCl	29.00	29.00	29.00	290.00	29.00	29.00
L-Methionine	4.48	4.48	4.48	44.80	4.48	4.48
L-Phenylalanine	5.00	5.00	5.00	50.00	5.00	5.00
L-Proline	11.50	11.50	11.50	115.00	11.50	11.50
L-Serine	10.50	10.50	10.50	105.00	10.50	10.50
L-Threonine	3.57	3.57	3.57	35.70	3.57	3.57
L-Tryptophan	0.60	0.60	0.60	6.00	0.60	0.60
L-Tyrosine • 2Na • $2H_2O$	2.61	2.61	2.61	26.10	2.61	2.61
L-Valine	3.50	3.50	3.50	35.00	3.50	3.50
VITAMINS						
D-Biotin	0.024	0.0241	0.0241	0.241	0.0241	0.0241
Choline Chloride	0.698	0.698	0.698	6.98	0.698	0.698
Folic Acid	1.32	1.32	1.32	13.20	1.32	1.32
myo-Inositol	0.541	0.541	0.541	5.41	0.541	0.541
Niacinamide	0.615	0.615	0.615	6.15	0.615	0.615
D-Pantothenic Acid (hemicalcium salt)	0.715	0.715	0.715	7.15	0.715	0.715
Pyridoxine • HCl	0.206	0.206	0.206	2.06	0.206	0.206
Riboflavin	0.376	0.376	0.376	3.76	0.376	0.376
Thiamine • HCl	1.00	1.00	1.00	10.00	1.00	1.00
Vitamin B-12	1.36	1.36	1.36	13.60	1.36	1.36
INORGANIC SALTS						
Calcium Chloride (anhydrous)	33.30	33.30	33.30	333.00	33.30	33.30
Cupric Sulfate (anhydrous)	0.0016	0.0016	0.0016	0.016	0.0016	0.0016
Ferrous Sulfate • $7H_2O$	0.834	0.834	0.834	8.34	0.834	0.834
Magnesium Sulfate (anhydrous)	74.60	74.60	74.60	746.00	74.60	74.60
Potassium Chloride	285.00	285.00	285.00	2850.00	285.00	285.00
Potassium Phosphate Monobasic (anhydrous)	83.00	83.00	83.00	830.00	83.00	83.00
Sodium Bicarbonate	1200.00	1200.00	--	--	--	--
Sodium Chloride	7400.00	7400.00	7400.00	74000.00	7400.00	7400.00
Sodium Phosphate Dibasic (anhydrous)	153.20	153.20	153.20	1532.00	153.20	153.20
Zinc Sulfate • $7H_2O$	0.0288	0.0288	0.0288	0.288	0.0288	0.0288
OTHER						
D-Glucose	1100.00	1100.00	1100.00	11000.00	1100.00	1100.00
HEPES	--	--	4766.00	--	--	--
Hypoxanthine • Na	4.74	4.74	4.74	47.40	--	4.74
Phenol Red (sodium)	1.20	1.20	1.20	12.00	1.20	1.20
Pyruvic Acid • Na	110.00	110.00	110.00	110.00	110.00	110.00
Thioctic Acid	0.20	0.20	0.20	2.00	0.20	0.20
Thymidine	0.73	0.73	0.73	7.30	0.73	0.73

Ham's F-12 Nutrient Mixture and Kaighn's Modification (F12K) (Modified)

F12K is a modification of Ham's F-12 and Coon's F-12 with increased amino acid and pyruvate concentrations. Additionally, the salts have been modified (Konigsbergs). F12K has been developed for culturing differentiated rat and chicken cells, as well as, primary human liver cells.

COMPONENT	1242254 1X Liquid mg/L	1242354 1X Liquid mg/L	1242454 Kaighn's 1X Liquid mg/L	1042122 Powder mg/L
AMINO ACIDS				
L-Alanine	8.90	8.90	18.00	8.90
L-Arginine • HCl	211.00	211.00	422.00	211.00
L-Asparagine • H_2O	15.00	15.00	30.00	15.00
L-Aspartic Acid	13.30	13.30	26.60	13.30
L-Cysteine • HCl • H_2O	35.12	35.12	70.00	35.12
L-Glutamic Acid	14.70	14.70	29.00	14.70
L-Glutamine	--	146.20	292.00	146.20
Glycine	7.50	7.50	15.00	7.50
L-Histidine • HCl • H_2O	21.00	21.00	45.80	21.00
L-Isoleucine	3.94	3.94	8.00	3.94
L-Leucine	13.10	13.10	26.20	13.10
L-Lysine • HCl	36.50	36.50	73.00	36.50
L-Methionine	4.48	4.48	9.00	4.48
L-Phenylalanine	4.96	4.96	10.00	4.96
L-Proline	34.50	34.50	69.00	34.50
L-Serine	10.50	10.50	21.00	10.50
L-Threonine	11.90	11.90	24.00	11.90
L-Tryptophan	2.04	2.04	4.00	2.04
L-Tyrosine • 2Na • $2H_2O$	7.84	7.84	13.50	7.84
L-Valine	11.70	11.70	23.40	11.70
VITAMINS				
D-Biotin	0.0073	0.0073	0.07	0.0073
Choline Chloride	13.96	13.96	13.96	13.96
Folic Acid	1.30	1.30	1.30	1.30
myo-Inositol	18.02	18.02	18.02	18.02
Niacinamide	0.037	0.037	0.037	0.037
D-Pantothenic Acid (hemicalcium salt)	0.48	0.48	0.48	0.48
Pyridoxine • HCl	0.062	0.062	0.062	0.062
Riboflavin	0.038	0.038	0.038	0.038
Thiamine • HCl	0.34	0.34	0.34	0.34
Vitamin B-12	1.36	1.36	1.36	1.36
INORGANIC SALTS				
Calcium Chloride (anhydrous)	33.30	33.30	102.00	33.30
Cupric Sulfate (anhydrous)	0.0016	0.0016	0.0016	0.0016
Ferrous Sulfate • $7H_2O$	0.834	0.834	0.834	0.834
Magnesium Sulfate (anhydrous)	72.20	72.20	192.00	72.20
Potassium Chloride	223.60	223.60	285.00	223.60
Potassium Phosphate Monobasic	--	--	59.00	--
Sodium Bicarbonate	1176.00	1176.00	2500.00	--
Sodium Chloride	7600.00	7600.00	7600.00	7600.00
Sodium Phosphate Dibasic (anhydrous)	142.00	142.00	115.50	142.00
Zinc Sulfate • $7H_2O$	0.863	0.863	0.144	0.863
OTHER				
D-Glucose	1802.00	1802.00	1260.00	1802.00
Hypoxanthine • Na	4.77	4.77	4.00	4.77
Methyl Linoleate	0.088	0.088	--	0.088
Phenol Red (sodium)	1.20	1.20	3.00	1.20
Putrescine • 2HCl	0.16	0.16	0.32	0.16
Pyruvic Acid • Na	110.00	110.00	220.00	110.00
Thioctic Acid	0.21	0.21	0.21	0.21
Thymidine	0.73	0.73	0.70	0.73

High Growth Enhancement Medium

ICN's High Growth Enhancement Medium is recommended for the growth of cells on microcarrier support matrices. It is a modification of DMEM where 3.6 g/L of fructose is substituted for 4.5 g/L of glucose, resulting in improved pH control and greater cell yields.

COMPONENT	1233754 1X Liquid mg/L	1033822 Powder mg/L
AMINO ACIDS		
L-Arginine • HCl	84.00	84.00
L-Cystine • 2Na • H_2O	60.38	60.38
L-Glutamine	--	584.00
Glycine	30.00	30.00
L-Histidine • HCl • H_2O	42.00	42.00
L-Isoleucine	104.80	104.80
L-Leucine	104.80	104.80
L-Lysine • HCl	146.20	146.20
L-Methionine	30.00	30.00
L-Phenylalanine	66.00	66.00
L-Serine	42.00	42.00
L-Threonine	95.20	95.20
L-Tryptophan	16.00	16.00
L-Tyrosine • 2Na • $2H_2O$	103.80	103.80
L-Valine	93.60	93.60
VITAMINS		
Choline Chloride	4.00	4.00
Folic Acid	4.00	4.00
myo-Inositol	7.00	7.00
Niacinamide	4.00	4.00
D-Pantothenic Acid (hemicalcium salt)	4.00	4.00
Pyridoxal • HCl	4.00	4.00
Riboflavin	0.40	0.40
Thiamine • HCl	4.00	4.00
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	264.90	264.90
Ferrous Nitrate • $9H_2O$	0.10	0.10
Magnesium Sulfate • $7H_2O$	200.00	200.00
Potassium Chloride	400.00	400.00
Sodium Bicarbonate	3700.00	--
Sodium Chloride	6400.00	6400.00
Sodium Phosphate Monobasic (anhydrous)	125.00	125.00
OTHER		
D-Fructose	3600.00	36.00
Phenol Red (sodium salt)	15.00	15.00
Pyruvic Acid • Na	110.00	110.00

Iscove's Modified Dulbecco's Medium (IMDM) (Modified)

Guilbert and Iscove's modification of DMEM was originally developed to culture precursor erythocyte and macrophage cells under completely defined, serum-free conditions. ICN's IMDM contains selenium, added amino acids and vitamins, sodium pyruvate, HEPES buffer and potassium nitrate in place of ferric nitrate. It has demonstrated proven performance in culturing a variety of cell types in both serum-free and serum-reduced conditions including murine B lymphocytes, hemopoietic bone marrow tissue, T lymphocytes and hybrid cells.

COMPONENT	1235854 1X Liquid mg/L	1235954 1X Liquid mg/L	1035722 Powder mg/L	1035522 Powder mg/L
AMINO ACIDS				
L-Alanine	25.00	25.00	25.00	25.00
L-Arginine • HCl	84.00	84.00	84.00	84.00
L-Asparagine • H_2O	28.40	28.40	28.40	28.40
L-Aspartic Acid	30.00	30.00	30.00	30.00
L-Cystine • H_2O	91.24	91.24	91.24	91.24
L-Glutamic Acid	75.00	75.00	75.00	75.00
L-Glutamine	584.00	584.00	584.00	584.00
Glycine	30.00	30.00	30.00	30.00
L-Histidine • HCl • H_2O	42.00	42.00	42.00	42.00
L-Isoleucine	105.00	105.00	105.00	105.00
L-Leucine	105.00	105.00	105.00	105.00
L-Lysine • HCl	146.00	146.00	146.00	146.00
L-Methionine	30.00	30.00	30.00	30.00
L-Phenylalanine	66.00	66.00	66.00	66.00
L-Proline	40.00	40.00	40.00	40.00
L-Serine	42.00	42.00	42.00	42.00
L-Threonine	95.00	95.00	95.00	95.00
L-Tryptophan	16.00	16.00	16.00	16.00
L-Tyrosine • 2Na • $2H_2O$	103.79	103.79	103.79	103.79
L-Valine	94.00	94.00	94.00	94.00
VITAMINS				
D-Biotin	0.013	0.013	0.013	0.013
Choline Chloride	4.00	4.00	4.00	4.00
Folic Acid	4.00	4.00	4.00	4.00
myo-Inositol	7.20	7.20	7.20	7.20
Niacinamide	4.00	4.00	4.00	4.00
D-Pantothenic Acid (hemicalcium salt)	4.00	4.00	4.00	4.00
Pyridoxine • HCl	4.00	4.00	4.00	4.00
Riboflavin	0.40	0.40	0.40	0.40
Thiamine • HCl	4.00	4.00	4.00	4.00
Vitamin B-12	0.013	0.013	0.013	0.013
INORGANIC SALTS				
Calcium Chloride (anhydrous)	165.00	165.00	165.00	165.00
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	97.70
Potassium Chloride	330.00	330.00	330.00	330.00
Potassium Nitrate	0.076	0.076	0.076	0.076
Sodium Bicarbonate	3024.00	2520.00	--	--
Sodium Chloride	4505.00	4505.00	4505.00	4505.00
Sodium Phosphate Monobasic • H_2O	125.00	125.00	125.00	125.00
Sodium Selenite	0.0173	0.0173	0.0173	0.0173
OTHER				
Bovine Serum Albumin	400.00	--	--	400.00
D-Glucose	4500.00	4500.00	4500.00	4500.00
HEPES	--	5958.00	5958.00	5958.00
Phenol Red (sodium salt)	15.00	15.00	15.00	15.00
Pyruvic Acid • Na	110.00	110.00	110.00	110.00
Soybean Lecithin	100.00	--	--	100.00
Transferrin, Human (purified)	1.00	--	--	1.00

Joklik's Minimum Essential Medium (JMEM) (Modified)

Joklik's modification of of MEM is specially suited for suspension cultures by optionally eliminating calcium from the formulation to enhance the growth of cells. ICN's JMEM only requires the addition of serum or serum-replacement products.

COMPONENT	1232354 1X Liquid mg/L	1032322 Powder mg/L
AMINO ACIDS		
L-Arginine • HCl	105.00	105.00
L-Cystine • 2Na • H_2O	31.48	31.48
L-Glutamine	--	294.00
L-Histidine • HCl • H_2O	41.90	41.90
L-Isoleucine	52.00	52.00
L-Leucine	52.00	52.00
L-Lysine • HCl	72.50	72.50
L-Methionine	15.00	15.00
L-Phenylalanine	32.00	32.00
L-Threonine	48.00	48.00
L-Tryptophan	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	54.48	54.48
L-Valine	46.00	46.00
VITAMINS		
Choline Chloride	1.00	1.00
Folic Acid	1.00	1.00
myo-Inositol	2.00	2.00
Niacinamide	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00
Pyridoxal • HCl	1.00	1.00
Riboflavin	0.10	0.10
Thiamine • HCl	1.00	1.00
INORGANIC SALTS		
Magnesium Sulfate • $7H_2O$	242.40	242.40
Potassium Chloride	400.00	400.00
Sodium Bicarbonate	2000.00	2000.00
Sodium Chloride	6500.00	6500.00
Sodium Phosphate Monobasic • H_2O	1327.00	1327.00
OTHER		
Dihydrostreptomycin Sulfate	50.00	50.00
D-Glucose	2000.00	2000.00
Penicillin G (sodium salt)	75000 IU	75000 IU
Phenol Red (sodium salt)	10.00	10.00

L-15 Medium (Modified)

L-15 was originally developed by Leibovitz for use in carbon dioxide (CO_2) free systems which only require sodim bicarbonate supplementation. In this modification, glucose has been replaced by galactose and buffering results from its complement of salts and free base forms of amino acids in place of sodium bicarbonate to help maintain pH control. L-15 supports established cell lines, like HEp-2 and LLC-MK_2, and primary explants of adult and embryonic human tissues when supplemented properly. Viruses may also be successfully cultured in this medium.

COMPONENT	1251054 1X Liquid mg/L	1251154 1X Liquid mg/L	1051122 Powder mg/L
AMINO ACIDS			
L-Alanine	225.00	225.00	225.00
L-Arginine	500.00	500.00	500.00
L-Asparagine • H_2O	250.00	250.00	250.00
L-Cysteine	120.00	120.00	120.00
L-Glutamine	--	300.00	300.00
Glycine	200.00	200.00	200.00
L-Histidine (free base)	250.00	250.00	250.00
L-Isoleucine	125.00	125.00	125.00
L-Leucine	125.00	125.00	125.00
L-Lysine • HCl	93.70	93.70	93.70
L-Methionine	75.00	75.00	75.00
L-Phenylalanine	125.00	125.00	125.00
L-Serine	200.00	200.00	200.00
L-Threonine	300.00	300.00	300.00
L-Tryptophan	20.00	20.00	20.00
L-Tyrosine • Na • $2H_2O$	432.40	432.40	432.40
L-Valine	100.00	100.00	100.00
VITAMINS			
Choline Chloride	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00	1.00
Pyridoxine • HCl	1.00	1.00	1.00
Riboflavin Phosphate • Na • $2H_2O$	0.1076	0.1076	0.1076
Thiamine Monophosphate • HCl	1.00	1.00	1.00
INORGANIC SALTS			
Calcium Chloride • $2H_2O$	185.50	185.50	185.50
Magnesium Sulfate • $7H_2O$	400.00	400.00	400.00
Potassium Chloride	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	60.00	60.00	60.00
Sodium Chloride	8000.00	8000.00	8000.00
Sodium Phosphate Dibasic (anhydrous)	190.00	190.00	190.00
OTHER			
D-Galactose	900.00	900.00	900.00
Phenol Red (sodium)	10.00	10.00	10.00
Pyruvic Acid • Na	550.00	550.00	550.00

McCoy's 5A Medium (Modified)

McCoy's 5A medium, as modified by Iwakata and Grace, is identical to RPMI 1629. Originally in 1959, McCoy and his colleagues described the amino acid requirements for the in vitro culturing of Novikoff Hepatoma cells. Basal Medium 5A was modified to create what is known as McCoy's 5A Medium for these investigations. Hsu and Kellogg further demonstrated the use of this medium to support the growth of primary cultures derived from normal bone marrow, testes, mouse kidney, skin, gingiva, rat embryo and other tissues. ICN's medium is additionally suited for the propagation of leukocytes, biopsy tisses and the most demanding primary and continuous cell types. It is also available as modified by Park and Terasaki.

COMPONENT	1255254 1X Liquid mg/L	1255354 1X Liquid mg/L	1692154 1X Liquid mg/L	1055122 Powder mg/L
AMINO ACIDS				
L-Alanine	13.36	13.36	13.36	13.36
L-Arginine • HCl	42.14	42.14	42.14	42.14
L-Asparagine • H_2O	45.03	45.03	45.03	45.03
L-Aspartic Acid	19.97	19.97	19.97	19.97
L-Cysteine • HCl • H_2O	35.14	35.14	31.53	35.14
L-Glutamic Acid	22.10	22.10	22.07	22.10
L-Glutamine	--	219.20	--	219.20
Glycine	7.50	7.50	7.51	7.50
L-Histidine • HCl • H2O	20.96	20.96	20.96	20.96
Hydroxy-L-proline	19.70	19.70	19.67	19.70
L-Isoleucine	39.36	39.36	39.36	39.36
L-Leucine	39.36	39.36	39.36	39.36
L-Lysine • HCl	36.54	36.54	36.54	36.54
L-Methionine	14.92	14.92	14.92	14.92
L-Phenylalanine	16.52	16.52	16.52	16.52
L-Proline	17.30	17.30	17.27	17.30
L-Serine	26.30	26.30	26.28	26.30
L-Threonine	17.90	17.90	17.87	17.90
L-Tryptophan	3.10	3.10	3.06	3.10
L-Tyrosine • 2Na • $2H_2O$	26.12	26.12	26.12	26.12
L-Valine	17.60	17.60	17.57	17.60
VITAMINS				
p-Aminobenzoic Acid	1.00	1.00	1.00	1.00
Ascorbic Acid	0.50	0.50	0.50	0.50
D-Biotin	0.20	0.20	0.20	0.20
Choline Chloride	5.00	5.00	5.00	5.00
Folic Acid	10.00	10.00	10.00	10.00
myo-Inositol	36.00	36.00	36.00	36.00
Niacinamide	0.50	0.50	0.50	0.50
Nicotinic Acid	0.50	0.50	0.50	0.50
D-Pantothenic Acid (hemicalcium salt)	0.20	0.20	0.20	0.20
Pyridoxal • HCl	0.50	0.50	0.50	0.50
Pyridoxine • HCl	0.50	0.50	0.50	0.50
Riboflavin	0.20	0.20	0.20	0.20
Thiamine • HCl	0.20	0.20	0.20	0.20
Vitamin B-12	2.00	2.00	2.00	2.00
INORGANIC SALTS				
Calcium Chloride (anhydrous)	100.00	100.00	100.00	100.00
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	97.70
Potassium Chloride	400.00	400.00	400.00	400.00
Sodium Bicarbonate	2200.00	2200.00	--	--
Sodium Chloride	6460.00	6460.00	6460.00	6460.00
Sodium Phosphate Monobasic • H_2O	580.00	580.00	580.00	580.00
OTHER				
Dihydrostreptomycin Sulfate	--	--	200.00	--
Fetal Bovine Serum	--	--	5 ml	--
Gentamicin	--	--	16.00	--
D-Glucose	3000.00	3000.00	3000.00	3000.00
Glutathione (reduced)	0.50	0.50	0.50	0.50
HEPES	--	--	2.86	--
Penicillin G (sodium salt)	--	--	200,000 IU	--
Peptone	600.00	600.00	600.00	600.00
Phenol Red (sodium)	10.00	10.00	10.00	10.00

Medium 199 (Modified)

Medium 199, originally described by Morgan and his colleagues (1950), is a completely defined nutrional source for cell culture. Their investigations demonstrated that cell growth could be measured in this medium. It has broad species applicability including the culturing of non-transformed cell types. It may be used for vacccine production and the *in vitro* cultivation of rat lens tissues and primary mouse pancreatic epithelial explants. It is available with either Earle's or Hanks' salts.

COMPONENT	1220254 1X Liquid mg/L	1220354 1X Liquid mg/L	1220454 1X Liquid mg/L	1223254 1X Liquid mg/L	1223354 1X Liquid mg/L	1420054 10X Liquid mg/L	1423054 10X Liquid mg/L	1020122 Powder mg/L	1023122 Powder mg/L
AMINO ACIDS									
L-Alanine	25.00	25.00	25.00	25.00	25.00	250.00	250.00	25.00	25.00
L-Arginine • HCl	70.00	70.00	70.00	70.00	70.00	700.00	700.00	70.00	70.00
L-Aspartic Acid	30.00	30.00	30.00	30.00	30.00	300.00	300.00	30.00	30.00
L-Cysteine • HCl • H_2O	0.11	0.11	0.11	0.11	0.11	1.10	1.10	0.11	1.10
L-Cystine • 2HCl	26.00	26.00	26.00	26.00	26.00	260.00	260.00	26.00	26.00
L-Glutamic Acid	66.82	66.82	66.82	66.82	66.82	668.20	668.20	66.82	66.82
L-Glutamine	--	100.00	--	--	100.00	--	--	100.00	100.00
Glycine	50.00	50.00	50.00	50.00	50.00	500.00	500.00	50.00	50.00
L-Histidine • HCl • H_2O	21.88	21.88	21.88	21.88	21.88	218.80	218.80	21.88	21.88
Hydroxy-L-proline	10.00	10.00	10.00	10.00	10.00	100.00	100.00	10.00	10.00
L-Isoleucine	20.00	20.00	20.00	20.00	20.00	200.00	200.00	20.00	20.00
L-Leucine	60.00	60.00	60.00	60.00	60.00	600.00	600.00	60.00	60.00
L-Lysine • HCl	70.00	70.00	70.00	70.00	70.00	700.00	700.00	70.00	70.00
L-Methionine	15.00	15.00	15.00	15.00	15.00	150.00	150.00	15.00	15.00
L-Phenylalanine	25.00	25.00	25.00	25.00	25.00	250.00	250.00	25.00	25.00
L-Proline	40.00	40.00	40.00	40.00	40.00	400.00	400.00	40.00	40.00
L-Serine	25.00	25.00	25.00	25.00	25.00	250.00	250.00	25.00	25.00
L-Threonine	30.00	30.00	30.00	30.00	30.00	300.00	300.00	30.00	30.00
L-Tryptophan	10.00	10.00	10.00	10.00	10.00	100.00	100.00	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	57.66	57.66	57.66	57.66	57.66	576.60	576.60	57.66	57.66
L-Valine	25.00	25.00	25.00	25.00	25.00	250.00	250.00	25.00	25.00
VITAMINS									
p-Aminobenzoic Acid	0.05	0.05	0.05	0.05	0.05	0.50	0.50	0.05	0.05
Ascorbic Acid • Na	0.05	0.05	0.05	0.05	0.05	0.50	0.50	0.05	0.05
D-Biotin	0.01	0.01	0.01	0.01	0.01	0.10	0.10	0.01	0.01
Calciferol	0.10	0.10	0.10	0.10	0.10	1.00	1.00	0.10	0.10
Choline Chloride	0.50	0.50	0.50	0.50	0.50	5.00	5.00	0.50	0.50
Folic Acid	0.01	0.01	0.01	0.01	0.01	0.10	0.10	0.01	0.01
myo-Inositol	0.05	0.05	0.05	0.05	0.05	0.50	0.50	0.05	0.05
Menadione (sodium Bisulfite)	0.019	0.019	0.019	0.019	0.019	0.19	0.19	0.019	0.019
Niacinamide	0.025	0.025	0.025	0.025	0.025	0.25	0.25	0.025	0.025
Nicotinic Acid	--	--	--	0.025	0.025	--	0.25	--	0.025
D-Pantothenic Acid (hemicalcium salt)	0.01	0.01	0.01	0.01	0.01	0.10	0.10	0.01	0.01
Pyridoxal • HCl	--	--	--	0.025	0.025	--	0.25	--	0.025
Pyridoxine • HCl	0.05	0.05	0.05	0.025	0.025	0.50	0.25	0.05	0.05
Retinol Acetate	0.14	0.14	0.14	0.14	0.14	1.40	1.40	0.14	0.14
Riboflavin	0.01	0.01	0.01	0.01	0.01	0.10	0.10	0.01	0.01
DL-α-Tocopherol Phosphate • Na	0.01	0.01	0.01	0.011	0.011	0.10	0.11	0.01	0.01
Thiamine • HCl	0.01	0.01	0.01	0.01	0.01	0.10	0.10	0.01	0.01
INORGANIC SALTS									
Calcium Chloride • $2H_2O$	264.90	264.90	264.90	185.50	185.50	2649.00	1855.00	264.90	185.50
Ferric Nitrate • $6H_2O$	0.72	0.72	0.72	0.72	0.72	7.20	7.20	0.72	0.72
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	--	--	977.00	--	97.70	--
Magnesium Sulfate • $7H_2O$	--	--	--	200.00	200.00	--	2000.00	--	200.00
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	4000.00	4000.00	400.00	400.00
Potassium Phosphate Monobasic	--	--	--	60.00	60.00	--	600.00	--	60.00
Sodium Acetate (anhydrous)	50.00	50.00	50.00	50.00	50.00	500.00	500.00	50.00	50.00
Sodium Bicarbonate	2200.00	2200.00	--	350.00	350.00	--	--	--	--
Sodium Chloride	6800.00	6800.00	6800.00	8000.00	8000.00	68000.00	80000.00	6800.00	8000.00
Sodium Phosphate Monobasic (anhydrous)	140.00	140.00	140.00	47.50	47.50	1400.00	475.00	140.00	47.50
OTHER									
Adenine Sulfate	10.00	10.00	10.00	10.98	10.98	100.00	109.80	10.00	10.98
Adenosine 5′-Monophosphate • $2H_2O$	--	--	--	0.2104	0.2104	--	2.104	--	0.2104
Adenosine 5′-Triphosphate • 2Na	1.00	1.00	1.00	1.098	1.098	10.00	10.98	1.00	1.098
Adenylic Acid	0.20	0.20	0.20	--	--	2.00	--	0.20	--
Cholesterol	0.20	0.20	0.20	0.20	0.20	2.00	2.00	0.20	0.20
2-Deoxyribose	0.50	0.50	0.50	0.50	0.50	5.00	5.00	0.50	0.50
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	10000.00	10000.00	1000.00	1000.00
Glutathione (reduced)	0.05	0.05	0.05	0.05	0.05	0.50	0.50	0.05	0.05
Guanine • HCl	0.30	0.30	0.30	0.30	0.30	3.00	3.00	0.30	0.30
HEPES	--	--	4766.00	--	--	--	--	--	--
Hypoxanthine • Na	0.354	0.354	0.35	0.30	0.30	3.54	3.00	0.354	0.30
Phenol Red (sodium)	10.00	10.00	10.00	17.00	17.00	100.00	170.00	10.00	17.00
D-Ribose	0.50	0.50	0.50	0.50	0.50	5.00	5.00	0.50	0.50
Thymine	0.30	0.30	0.30	0.30	0.30	3.00	3.00	0.30	0.30
Tween 80	5.00	5.00	5.00	20.00	20.00	50.00	200.00	5.00	20.00
Uracil	0.30	0.30	0.30	0.30	0.30	3.00	3.00	0.30	0.30
Xanthine • Na	0.30	0.30	0.30	0.30	0.30	3.00	3.00	0.34	0.30

Minimum Essential Medium Eagle (MEM) (Modified)

MEM, originally prepared by Harry Eagle, is one of the most popular cell culture media. Upon his attempts to cultivate normal mammalian fibroblasts and certain HeLa cell subtypes, it was revealed that the nutritional needs of these cell types could not be met by BME. Further studies led to the development of MEM incorporating specific modifications such as higher amino acid concentrations for the cultivation of fastidious cells. ICN's MEM may be used to support the growth of cells in monolayers, in suspension and wide variety of other cell types with proper supplementation. ICN offers MEM with either Earle's or Hanks' salts.

COMPONENT	1210254 1X Liquid mg/L	1210354 1X Liquid mg/L	1210454 1X Liquid mg/L	1210654 1X Liquid mg/L	1213254 1X Liquid mg/L	1213454 1X Liquid mg/L	1216254 1X Liquid mg/L	1217654 1X Liquid mg/L
AMINO ACIDS								
L-Arginine • HCl	126.40	126.40	126.40	126.40	126.40	126.40	126.40	126.40
L-Cystine • 2HCl	31.20	31.20	31.20	31.20	--	--	31.20	31.20
L-Cystine • 2Na • H_2O	--	--	--	--	30.22	30.22	--	--
L-Glutamine	--	292.00	--	--	--	--	--	--
L-Histidine • HCl • H_2O	41.90	41.90	41.90	41.90	41.90	41.90	41.90	41.90
L-Isoleucine	52.50	52.50	52.50	52.50	52.50	52.50	52.50	52.50
L-Leucine	52.50	52.50	52.50	52.50	52.50	52.50	52.50	52.50
L-Lysine • HCl	72.50	72.50	72.50	72.50	73.06	73.06	72.50	72.50
L-Methionine	15.00	15.00	15.00	15.00	14.90	14.90	15.00	15.00
L-Phenylalanine	32.50	32.50	32.50	32.50	33.02	33.02	32.50	32.50
L-Threonine	47.60	47.60	47.60	47.60	47.60	47.60	47.60	47.60
L-Tryptophan	10.00	10.00	10.00	10.00	10.20	10.20	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	51.90	51.90	51.90	51.90	51.90	51.90	51.90	51.90
L-Valine	46.80	46.80	46.80	46.80	46.90	46.90	46.80	46.80
VITAMINS								
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pyridoxal • HCl	--	--	--	--	1.00	1.00	--	--
Pyridoxine • HCl	1.00	1.00	1.00	1.00	--	--	1.00	1.00
Riboflavin	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
INORGANIC SALTS								
Calcium Chloride (anhydrous)	200.00	200.00	200.00	200.00	--	--	--	--
Calcium Chloride • $2H_2O$	--	--	--	--	185.50	185.50	--	--
Magnesium Chloride • $6H_2O$	--	--	--	--	--	--	200.00	--
Magnesium Sulfate (anhydrous)	97.70	97.70	97.70	97.70	--	--	--	--
Magnesium Sulfate • $7H_2O$	--	--	--	--	200.00	200.00	--	--
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	--	--	--	--	60.00	60.00	--	--
Sodium Bicarbonate	2000.00	2000.00	2000.00	850.00	350.00	--	2000.00	850.00
Sodium Chloride	6800.00	6800.00	6800.00	6800.00	8000.00	8000.00	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	140.00	140.00	140.00	140.00	--	--	140.00	140.00
Sodium Phosphate Dibasic (anhydrous)	--	--	--	--	47.50	47.50		
OTHER								
Gentamicin Sulfate	--	--	--	--	--	--	--	--
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
HEPES	--	--	4766.00	--	--	4766.00	--	--
Phenol Red (sodium)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00

Minimum Essential Medium Eagle (MEM) (Modified)

COMPONENT	1212754 1X Liquid mg/L	1231254 1X Liquid mg/L	1610554 1X Liquid mg/L	1641454 1X Liquid mg/L	1622054 1X Liquid mg/L	1622154 1X Liquid mg/L	1622254 1X Liquid mg/L
AMINO ACIDS							
L-Alanine	8.90	25.00	--	--	--	--	--
L-Arginine • HCl	126.40	126.40	126.40	126.40	--	126.40	126.40
L-Asparagine • H_2O	15.00	50.00	--	--	--	--	--
L-Aspartic Acid	13.30	30.00	--	--	--	--	--
L-Cysteine • HCl • H_2O	--	89.74	--	--	--	--	--
L-Cystine • 2HCl	31.20	30.22	31.20	--	--	--	--
L-Cystine • 2Na • H_2O	--	--	--	--	30.22	30.22	30.22
L-Glutamic Acid	14.70	75.00	--	--	--	--	--
L-Glutamine	--	--	292.00	--	--	--	--
Glycine	7.50	50.00	--	--	--	--	--
L-Histidine • HCl • H_2O	41.90	41.90	41.90	41.90	41.90	41.90	41.90
L-Isoleucine	52.50	52.50	52.50	52.50	52.50	52.50	52.50
L-Leucine	52.50	52.50	52.50	52.50	52.50	--	52.50
L-Lysine • HCl	72.50	73.06	72.50	73.06	73.06	73.06	73.06
L-Methionine	15.00	14.90	15.00	--	14.90	14.90	--
L-Phenylalanine	32.50	33.02	32.50	33.02	33.02	33.02	33.02
L-Proline	11.50	40.00	2.00	--	--	--	--
L-Serine	10.50	25.00	--	--	--	--	--
L-Threonine	47.60	47.60	47.60	47.64	47.64	47.64	47.64
L-Tryptophan	10.00	10.20	10.00	10.20	10.20	10.20	10.20
L-Tyrosine • 2Na • $2H_2O$	51.90	51.90	51.90	51.90	51.90	51.90	51.90
L-Valine	46.80	46.90	46.80	46.90	46.90	46.90	46.90
VITAMINS							
L-Ascorbic Acid	--	50.00	--	--	--	--	--
D-Biotin	--	0.10	--	--	--	--	--
Choline Chloride	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pyridoxal • HCl	--	--	--	1.00	1.00	1.00	1.00
Pyridoxine • HCl	1.00	1.00	1.00	--	--	--	--
Riboflavin	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin B_{12}	--	1.36	--	--	--	--	
INORGANIC SALTS							
Calcium Chloride (anhydrous)	200.00	264.90	200.00	--	--	--	--
Calcium Chloride • $2H_2O$	--	--	--	264.90	264.90	264.90	264.90
Magnesium Chloride • $6H_2O$	--	--	--	--	--	--	--
Magnesium Sulfate (anhydrous)	97.70	200.00	97.70	97.70	97.70	97.70	97.70
Magnesium Sulfate • $7H_2O$	--	--	--	--	--	--	--
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	--	--	--	--	--	--	--
Sodium Bicarbonate	850.00	2000.00	2200.00	2000.00	2000.00	2000.00	2000.00
Sodium Chloride	6800.00	6800.00	6800.00	6800.00	6800.00	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	140.00	140.00	140.00	140.00	140.00	140.00	140.00
Sodium Phosphate Dibasic (anhydrous)	--	--	--	--	--	--	--
OTHER							
Gentamicin Sulfate	--	--	0.05	--	--	--	--
D-Glucose	1000.00	1000.00	1000.00	--	--	--	--
HEPES	--	--	--	--	--	--	--
Penicillin G (sodium salt)	200 IU	--	--	--	--	--	--
Phenol Red • Na	10.00	10.00	--	10.00	10.00	10.00	10.00
Pyruvic Acid • Na	--	110.00	--	--	--	--	--
Streptomycin Sulfate	0.10	--	--	--	--	--	--
Thioctic Acid	--	0.20	--	--	--	--	--

Minimum Essential Medium Eagle (MEM) (Modified)

COMPONENT	1622754 1X Liquid mg/L	1410054 10X Liquid mg/L	1417054 10X Liquid mg/L	1010122 Powder mg/L	1010522 Powder mg/L	1031122 Powder mg/L	1012122 Powder mg/L
AMINO ACIDS							
L-Alanine	--	--	--	--	--	25.00	8.90
L-Arginine • HCl	126.40	1264.00	1264.00	126.40	126.40	126.40	126.40
L-Asparagine • H_2O	--	--	--	--	--	50.00	15.00
L-Aspartic Acid	--	--	--	--	--	30.00	13.30
L-Cysteine • HCl • H_2O	--	--	--	--	--	100.00	--
L-Cystine • 2HCl	--	312.00	312.00	31.20	31.20	31.20	31.20
L-Cystine • 2Na • H_2O	30.22	--	--	--	--	--	--
L-Glutamic Acid	--	--	--	--	--	75.00	14.70
L-Glutamine	--	--	--	292.00	292.00	292.00	292.00
Glycine	--	--	--	--	--	50.00	7.50
L-Histidine • HCl • H_2O	41.90	419.00	419.00	41.90	41.90	41.90	41.90
L-Isoleucine	52.50	525.00	525.00	52.50	52.50	52.50	52.50
L-Leucine	52.50	525.00	525.00	52.50	52.50	52.50	52.50
L-Lysine • HCl	73.06	725.00	725.00	72.50	72.50	72.50	72.50
L-Methionine	14.90	150.00	150.00	15.00	15.00	15.00	15.00
L-Phenylalanine	33.02	325.00	325.00	32.50	32.50	32.50	32.50
L-Proline	--	--	--	--	--	40.00	11.50
L-Serine	--	--	--	--	--	25.00	10.50
L-Threonine	47.64	476.00	478.00	47.60	47.60	47.60	47.60
L-Tryptophan	10.20	100.00	100.00	10.00	10.00	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	51.90	519.00	519.00	51.90	51.90	51.90	51.90
L-Valine	46.90	469.00	468.00	46.80	46.80	46.80	46.80
VITAMINS							
L-Ascorbic Acid	--	--	--	--	--	50.00	--
D-Biotin	--	--	--	--	--	0.10	--
Choline Chloride	1.00	10.00	10.00	1.00	1.00	1.00	1.00
Folic Acid	1.00	10.00	10.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	20.00	20.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	10.00	10.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	10.00	10.00	1.00	1.00	1.00	1.00
Pyridoxal • HCl	1.00	--	--	--	--	--	--
Pyridoxine • HCl	--	10.00	10.00	1.00	1.00	1.00	1.00
Riboflavin	0.10	1.00	1.00	0.10	0.10	0.10	0.10
Thiamine • HCl	1.00	10.00	10.00	1.00	1.00	1.00	1.00
Vitamin B-12	--	--	--	--	--	1.36	--
INORGANIC SALTS							
Calcium Chloride • $2H_2O$	264.90	--	--	--	--	--	--
Calcium Chloride (anhydrous)	--	2000.00	--	200.00	200.00	200.00	200.00
Magnesium Sulfate (anhydrous)	200.00	977.00	--	97.70	97.70	97.70	97.70
Potassium Chloride	400.00	4000.00	4000.00	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	--	--	--	--	--	--	--
Sodium Bicarbonate	2000.00	--	--	--	--	--	--
Sodium Chloride	6800.00	68000.00	68000.00	6800.00	6800.00	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	--	1400.00	1400.00	140.00	140.00	140.00	140.00
Sodium Phosphate Dibasic (anhydrous)	--	--	--	--	--	--	--
Succinic Acid (free acid)	--	--	--	--	--	--	--
OTHER							
D-Glucose	--	10000.00	10000.00	1000.00	1000.00	1000.00	1000.00
HEPES	--	--	--	--	4766.00	--	--
Phenol Red • Na	10.00	100.00	100.00	10.00	10.00	10.00	10.00
Pyruvic Acid • Na	--	--	--	--	--	110.00	--
Thioctic Acid	--	--	--	--	--	0.20	--

Minimum Essential Medium Eagle (MEM) (Modified)

COMPONENT	1013122 Powder mg/L	1013522 Powder mg/L	1017122 Powder mg/L	1110022 Auto-Pow™ Powder mg/L	1111022 Auto-Pow™ Powder mg/L	1117022 Auto-Pow™ Powder mg/L
AMINO ACIDS						
L-Arginine • HCl	126.40	126.40	126.40	126.40	126.40	126.40
L-Cystine • 2HCl	--	--	31.20	31.20	31.20	31.20
L-Cystine • 2Na • H_2O	30.22	30.22	--	--	--	--
L-Glutamine	292.30	292.00	292.00	--	--	--
L-Histidine • HCl • H_2O	41.90	41.90	41.90	41.90	41.90	41.90
L-Isoleucine	52.50	52.50	52.50	52.50	52.50	52.50
L-Leucine	52.50	52.50	52.50	52.50	52.50	52.50
L-Lysine • HCl	73.06	73.06	72.50	72.50	72.50	72.50
L-Methionine	14.90	14.90	15.00	15.00	15.00	15.00
L-Phenylalanine	33.02	33.02	32.50	32.50	32.50	32.50
L-Threonine	47.64	47.64	47.80	47.60	47.60	47.80
L-Tryptophan	10.20	10.20	10.00	10.00	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	51.90	51.90	51.90	51.90	51.90	51.90
L-Valine	46.90	46.90	46.80	46.80	46.80	46.80
VITAMINS						
Choline Bitartrate	--	--	--	1.80	1.80	1.80
Choline Chloride	1.00	1.00	1.00	--	--	--
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00
myo-Inositol	2.00	2.00	2.00	2.00	2.00	2.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00	1.00	1.00	1.00	1.00
Pyridoxal • HCl	1.00	1.00	--	--	--	--
Pyridoxine • HCl	--	--	1.00	1.00	1.00	1.00
Riboflavin	0.10	0.10	0.10	0.10	0.10	0.10
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00
INORGANIC SALTS						
Calcium Chloride (anhydrous)	185.50	185.50	--	200.00	200.00	--
Magnesium Sulfate (anhydrous)	--	--	--	97.70	97.70	--
Magnesium Sulfate • $7H_2O$	200.00	200.00	--	--	--	--
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	60.00	60.00	--	--	--	--
Sodium Chloride	--	--	6800.00	6800.00	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	--	--	140.00	140.00	140.00	140.00
Sodium Phosphate Dibasic (anhydrous)	47.50	47.50	--	--	--	--
Sodium Succinate • $6H_2O$	--	--	--	100.00	100.00	100.00
Succinic Acid (free acid)	--	--	--	75.00	75.00	75.00
OTHER						
D-Glucose	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
HEPES	--	4766.00	--	--	--	--
Phenol Red • Na	10.00	10.00	10.00	10.00	--	10.00

Mitsuhashi and Maramorosch Insect Medium (MM)

This medium, developed by Mitsuhashi and Maramorosch, serves to establish and maintain cells derived from leafhoppers. It has been used in a broad variety of applications for the rapid growth and propagation of cells derived from insects when properly supplemented with 5-20% fetal bovine serum. It is most commonly employed for the cultivation of several mosquito cell lines.

COMPONENT	2700354 1X Liquid mg/L
INORGANIC SALTS	
Calcium Chloride • $2H_2O$	250.00
Magnesium Chloride • $6H_2O$	125.00
Potassium Chloride	250.00
Sodium Chloride	8750.00
Sodium Phosphate Monobasic • H_2O	250.00
OTHER	
D-Glucose	5000.00
Lactalbumin Hydrolysate	8125.00
Yeast Extract	6250.00

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1. Mitsuhashi, J. (1982) Media for Insect Cell Cultures. In: Advances in Cell Culture Vol. 2, K. Maramorosch ed., Academic Press, NY., pp. 133-196.

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3. Mitsuhashi, J. (1983) A Continuous Cell Line Derived from Fat Bodies of the Common Armyworm, Leucania separata (Lepidoptera: Nocuidae). Appl. Entomol. Zool., **18**:533.

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5. Sohi, S.S. (1980) The Effect of pH and Osmotic Pressure on the Growth and Survival of Three Lepidoptera Cell Lines. In: Invertebrate Systems In Vitro, E. Kurstak, K. Maramorosch and A. Dubendorfer eds., Elsevier/North-Holland Biomedical Press, Amsterdam, pp. 35-43.

Murashige and Skoog Plant Medium

The following media have been developed for the cultivation of a variety of plant cell types. In addition, plant salt mixture and Gamborg's B5 medium formulations are included.

COMPONENT	2610022 Powder mg/L	2600022 Powder mg/L	26330-2 Powder mg/L	2613022 Gamborg's B5 Powder mg/L
INORGANIC SALTS				
Ammonium Nitrate	1650.00	1650.00	1650.00	--
Boric Acid	6.20	6.20	6.20	3.00
Calcium Chloride • $2H_2O$	439.80	439.80	439.80	150.00
Cobalt Chloride • $6H_2O$	0.025	0.025	0.025	0.025
Cupric Sulfate • $5H_2O$	0.025	0.025	0.025	0.025
Fe Na • EDTA	36.70	36.70	36.70	40.00
Magnesium Sulfate • $7H_2O$	370.60	370.60	370.60	250.00
Manganese Sulfate • $4H_2O$	22.30	22.30	22.30	13.20
Molybdic Acid (sodium salt) • $2H_2O$	0.25	0.25	0.25	0.25
Potassium Iodide	0.83	0.83	0.83	0.75
Potassium Nitrate	1900.00	1900.00	1900.00	3000.00
Potassium Phosphate Monobasic (anhydrous)	170.00	170.00	170.00	--
Sodium Phosphate Monobasic • H_2O	--	--	--	150.00
Zinc Sulfate • $7H_2O$	8.60	8.60	8.60	2.00
OTHER				
Glycine	2.00	--	--	--
Inositol	100.00	100.00	--	100.00
Nicotinic Acid	0.50	--	--	1.00
Pyridoxine • HCl	0.50	--	--	1.00
Sucrose	--	30000.00	--	--
Thiamine • HCl	0.10	0.40	--	10.00

NCTC 135 Medium

Originated by the Tissue Culture Section (National Cancer Institute), NCTC 135 is identical to NCTC 109 except for the absence of L-cysteine. It supports L929 cells under chemically defined, serum-free conditions. However, other cell lines have been successfully adapted to NCTC 135, especially when adding serum.

COMPONENT	1291354 1X Liquid mg/L
AMINO ACIDS	
L-Alanine	31.48
L-Arginine • HCl	31.16
L-Asparagine • H_2O	9.19
L-Aspartic Acid	9.91
L-Cystine • 2Na • H_2O	13.20
L-Glutamic Acid	8.26
L-Glutamine	135.70
Glycine	13.51
L-Histidine • HCl • H_2O	26.65
Hydroxy-L-proline	4.09
L-Isoleucine	18.04
L-Leucine	20.44
L-Lysine • HCl	38.43
L-Methionine	4.44
L-Ornithine • HCl	9.41
L-Phenylalanine	16.53
L-Proline	6.13
L-Serine	10.75
L-Taurine	4.18
L-Threonine	18.93
L-Tryptophan	17.50
L-Tyrosine • 2Na • $2H_2O$	23.70
L-Valine	25.00
VITAMINS	
p-Aminobenzoic Acid	0.125
L-Ascorbic Acid	50.00
D-Biotin	0.025
Calciferol	0.25
Choline Chloride	1.25
Folic Acid	0.025
myo-Inositol	0.125
Menadione (sodium bisulfite)	0.048
Niacinamide	0.0625
Nicotinic Acid	0.0625
D-Pantothenic Acid (hemicalcium salt)	0.025
Pyridoxal • HCl	0.0625
Pyridoxine • HCl	0.0625
Retinol Acetate	0.29
Riboflavin	0.025
Thiamine • HCl	0.025
DL-α-Tocopherol Phosphate • 2Na	0.02662
Vitamin B-12	10.00
INORGANIC SALTS	
Calcium Chloride • $2H_2O$	264.90
Magnesium Sulfate • $7H_2O$	204.80
Potassium Chloride	400.00
Sodium Acetate (anhydrous)	30.14
Sodium Bicarbonate	2200.00
Sodium Chloride	6800.00
Sodium Phosphate Monobasic • H_2O	140.00
OTHER	
L-Amino-n-butyric Acid	5.51
Cocarboxylase	1.00
Coenzyme A • 3Li	2.39
2′-Deoxyadenosine	10.00
2′-Deoxycytidine • HCl	10.00
2′-Deoxyguanosine • HCl	10.00
Flavin Adenine Dinucleotide • 2Na	1.056
D-Glucosamine • HCl	3.85
D-Glucose	1000.00
Glucuronate • Na	1.80
D-Glucuronolactone	1.80
Glutathione • Na	9.22
5′-Methylcytosine • HCl	0.10
β-NAD • H_2O	7.19
β-NADP • 2Na • H_2O	1.027
Phenol Red (sodium)	20.00
Thymidine	10.00
Tween 80	12.50
Uridine Triphosphate • Na	1.00

Ovum Culture Medium

ICN's Ovum Culture medium is specially suited for the *in vitro* development and cultivation of bovine morulae.

COMPONENT	1634754 1X Liquid mg/L
INORGANIC SALTS	
Calcium Chloride • $2H_2O$	132.50
Magnesium Chloride • $6H_2O$	100.00
Potassium Chloride	200.00
Potassium Phosphate Monobasic (anhydrous)	200.00
Sodium Chloride	8000.00
Sodium Phosphate Dibasic (anhydrous)	1150.00
OTHER	
BSA (fraction V)	4000.00
D-Glucose	1000.00
Kanamycin Sulfate	25.00
Phenol Red (sodium)	5.00
Pyruvic Acid • Na	36.00

RPMI 1640 Medium (Modified)

This medium was originally developed by Moore and his colleagues at Roswell Park Memorial Institute (RPMI). It was based on the RPMI 1630 line of media which utilized a bicarbonate buffering system and alterations in the amounts of amino acids and vitamins. RPMI 1640 has successfully been used for the cultivation of normal human and neoplastic leukocytes. It is now a popular general purpose medium when properly supplemented.

COMPONENT	1260254 1X Liquid mg/L	1260354 1X Liquid mg/L	1260454 1X Liquid mg/L	1260554 1X Liquid mg/L	1260654 1X Liquid mg/L	1260954 1X Liquid mg/L	1265254 1X Liquid mg/L	1265354 1X Liquid mg/L
AMINO ACIDS								
L-Arginine	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
L-Asparagine • H_2O	56.82	56.82	56.82	56.82	56.82	56.82	56.82	56.82
L-Aspartic Acid	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Cystine • 2HCl	65.20	65.20	65.20	65.20	65.20	65.20	65.20	65.20
L-Glutamic Acid	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Glutamine	--	300.00	--	--	300.00	--	--	--
Glycine	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
L-Histidine (free base)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Hydroxy-L-proline	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Isoleucine	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
L-Leucine	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
L-Lysine • HCl	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
L-Methionine	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
L-Phenylalanine	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
L-Proline	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Serine	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
L-Threonine	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Tryptophan	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
L-Tyrosine • 2Na • $2H_2O$	28.83	28.83	28.83	28.83	28.83	28.83	28.83	28.83
L-Valine	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
VITAMINS								
p-Aminobenzoic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Biotin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
myo-Inositol	35.00	35.00	35.00	35.00	35.00	35.00	35.00	--
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Pyridoxine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Riboflavin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin B-12	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
INORGANIC SALTS								
Calcium Nitrate • $4H_2O$	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Magnesium Sulfate (anhydrous)	48.80	48.80	48.80	48.80	48.80	48.80	48.80	48.80
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00
Sodium Bicarbonate	2000.00	2000.00	--	2000.00	2000.00	1000.00	1000.00	1000.00
Sodium Chloride	6000.00	6000.00	6000.00	4750.00	4750.00	6400.00	6400.00	6400.00
Sodium Phosphate Dibasic (anhydrous)	800.70	800.70	800.70	800.70	800.70	800.70	800.70	800.70
OTHER								
Dextrose	2000.00	2000.00	2000.00	2000.00	2000.00	2000.00	2000.00	2000.00
Glutathione (reduced)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HEPES	--	--	4766.00	5958.00	5958.00	5958.00	--	--
Phenol Red (sodium)	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00

RPMI 1640 Medium (Modified)

COMPONENT	1629154 1X Liquid mg/L	1629254 1X Liquid mg/L	1629754 1X Liquid mg/L	1646454 1X Liquid mg/L	1646754 1X Liquid mg/L	1646854 1X Liquid mg/L	1460054 10X Liquid mg/L	1060122 Powder mg/L	1060522 Powder mg/L
AMINO ACIDS									
L-Arginine	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00	200.00
L-Asparagine	56.82	56.82	56.82	56.82	56.82	56.82	56.82	56.82	56.82
L-Aspartic Acid	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Cystine • 2HCl	65.20	65.20	65.20	--	65.20	65.20	65.20	65.20	65.20
L-Glutamic Acid	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Glutamine	--	--	--	--	--	--	--	300.00	300.00
Glycine	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
L-Histidine (free base)	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Hydroxy-L-proline	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Isoleucine	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
L-Leucine	--	50.00	50.00	50.00	50.00	50.00	50.00	50.00	50.00
L-Lysine • HCl	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
L-Methionine	15.00	--	15.00	--	15.00	15.00	15.00	15.00	15.00
L-Phenylalanine	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
L-Proline	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Serine	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
L-Threonine	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
L-Tryptophan	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
L-Tyrosine • 2Na • 2H_2O	28.83	28.83	28.83	28.83	28.83	28.83	28.83	28.83	28.83
L-Valine	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
VITAMINS									
p-Aminobenzoic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Biotin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Choline Chloride	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Folic Acid	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
myo-Inositol	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00	35.00
Niacinamide	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Pyridoxine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Riboflavin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Thiamine • HCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vitamin B-12	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
INORGANIC SALTS									
Calcium Nitrate • 4H_2O	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Magnesium Sulfate (anhydrous)	48.80	48.80	48.80	48.80	48.80	48.80	48.80	48.80	48.80
Potassium Chloride	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00	400.00
Sodium Bicarbonate	1000.00	1000.00	850.00	2000.00	2000.00	2000.00	--	--	--
Sodium Chloride	6400.00	6400.00	6400.00	6000.00	6000.00	6000.00	6000.00	6000.00	6000.00
Sodium Phosphate Dibasic (anhydrous)	800.70	800.70	--	800.70	800.70	800.70	800.70	800.70	800.70
OTHER									
Dextrose	2000.00	2000.00	2000.00	2000.00	2000.00	--	2000.00	2000.00	2000.00
Glutathione (reduced)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
HEPES	--	--	--	--	--	--	--	--	5958.00
Phenol Red (sodium)	5.00	5.00	5.00	5.00	--	5.00	5.00	5.00	5.00

TC 100 Medium (Modified)

This medium was originally known as BML-TC/10 when first developed in the 1970's by Dr. Gardiner and Dr. Stockdale. It is a modification of Grace's formulation, and today, it is more commonly referred to as TC-100. It is optimized for the production of Autographica californica NPV virions by cells from *Spodoptera frugiperda* (fall armyworm). When additionally supplemented, it is proven effective for cultivating baculoviruses in a variety of lepidopteran species.

COMPONENT	2705553 1X Liquid mg/L
AMINO ACIDS	
L-Alanine	250.00
L-Arginine	611.10
L-Asparagine • H_2O	441.90
L-Aspartic Acid	388.90
L-Cysteine	24.44
L-Cystine • 2Na • H_2O	--
L-Glutamic Acid	666.70
L-Glutamine	666.70
Glycine	722.20
L-Histidine	3778.00
L-Isoleucine	55.56
L-Leucine	83.33
L-Lysine • HCl	700.00
L-Methionine	55.56
L-Phenylalanine	166.70
L-Proline	388.90
L-Serine	611.10
L-Threonine	200.00
L-Tryptophan	111.10
L-Tyrosine	55.56
L-Valine	111.10
VITAMINS	
p-Aminobenzoic Acid	0.02222
D-Biotin	0.01111
Folic Acid	0.02222
myo-Inositol	0.02222
Nicotinic Acid	0.02222
D-Pantothenic Acid (hemicalcium salt)	0.1222
Pyridoxine • HCl	0.02222
Riboflavin	0.02222
Thiamine • HCl	0.02222
Vitamin B-12	0.01111
INORGANIC SALTS	
Calcium Chloride • $2H_2O$	1467.00
Magnesium Chloride • $6H_2O$	2533.00
Magnesium Sulfate • $7H_2O$	3089.00
Potassium Chloride	3189.00
Sodium Bicarbonate	388.90
Sodium Phosphate Monobasic • H_2O	1121.00
OTHER	
D-Glucose	1111.00
Tryptose Broth	2889.00

Waymouth MB 752/1 Medium

Waymouth MB 752/1 was originally developed as a completely defined, serum-free medium for the cultivation of L-929 cells. L929 cell clones which have been properly conditioned to grow in either serum-supplemented or serum-free medium have been successfully cultivated over thirty passages in this medium with a doubling time of about 24 hours. Today, Waymouth MB 752/1 medium serves as a general purpose medium for fastidious cells such as carcinoma cells from pleural effusions, whole organ cultures and potential tumorigenic cell types before their *in vivo* assessment.

COMPONENT	1252254 1X Liquid mg/L	1052122 Powder mg/L
AMINO ACIDS		
L-Arginine • HCl	75.00	75.00
L-Aspartic Acid	60.00	60.00
L-Cysteine • HCl	80.77	80.77
L-Cystine • 2Na • H_2O	18.82	18.82
L-Glutamic Acid	150.00	150.00
L-Glutamine	--	350.00
Glycine	50.00	50.00
L-Histidine • HCl • H_2O	164.10	164.10
L-Isoleucine	25.00	25.00
L-Leucine	50.00	50.00
L-Lysine • HCl	240.00	240.00
L-Methionine	50.00	50.00
L-Phenylalanine	50.00	50.00
L-Proline	50.00	50.00
L-Threonine	75.00	75.00
L-Tryptophan	40.00	40.00
L-Tyrosine • 2Na • $2H_2O$	57.65	57.65
L-Valine	65.00	65.00
VITAMINS		
Ascorbic Acid • Na	17.50	17.50
D-Biotin	0.02	0.02
Choline Chloride	250.00	250.00
Folic Acid	0.40	0.40
myo-Inositol	1.00	1.00
Niacinamide	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00
Pyridoxine • HCl	1.00	1.00
Riboflavin	1.00	1.00
Thiamine • HCl	10.00	10.00
Vitamin B-12	0.20	0.20
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	120.00	120.00
Magnesium Sulfate • $7H_2O$	491.00	491.00
Potassium Chloride	150.00	150.00
Potassium Phosphate Monobasic (anhydrous)	80.00	80.00
Sodium Bicarbonate	2240.00	--
Sodium Chloride	6000.00	6000.00
Sodium Phosphate Dibasic (anhydrous)	300.00	300.00
OTHER		
D-Glucose	5000.00	5000.00
Glutathione (reduced)	15.00	15.00
Hypoxanthine	25.00	25.00
Phenol Red (sodium)	10.00	10.00

Williams' Medium E (Modified)

Isolated epithelial cells, as described in his sequential plating method by Williams, et al. in 1971, were originally cultivated in a rich medium known as Williams' Medium D. From these early newborn animal studies, Williams and Gunn developed a subsequent medium, Williams' Medium E medium, for the long-term cultivation of adult rat liver epithelial cells.

COMPONENT	1250254 1X Liquid mg/L	1050122 Powder mg/L
AMINO ACIDS		
L-Alanine	90.00	90.00
L-Arginine • HCl	60.50	60.50
L-Asparagine • H_2O	20.00	20.00
L-Aspartic Acid	30.00	30.00
L-Cysteine • HCl	52.05	52.05
L-Cystine • 2Na • H_2O	25.29	25.29
L-Glutamic Acid	50.00	50.00
L-Glutamine	--	292.00
Glycine	50.00	50.00
L-Histidine • HCl • H_2O	20.30	20.30
L-Isoleucine	50.00	50.00
L-Leucine	75.00	75.00
L-Lysine • HCl	87.50	87.50
L-Methionine	15.00	15.00
L-Phenylalanine	25.00	25.00
L-Proline	30.00	30.00
L-Serine	10.00	10.00
L-Threonine	40.00	40.00
L-Tryptophan	10.00	10.00
L-Tyrosine • 2Na • $2H_2O$	50.45	50.45
L-Valine	50.00	50.00
VITAMINS		
Ascorbic Acid	2.00	2.00
D-Biotin	0.50	0.50
Calciferol	1.00	1.00
Choline Chloride	1.50	1.50
Folic Acid	1.00	1.00
myo-Inositol	2.00	2.00
Menadione (sodium bisulfite)	0.01	0.01
Niacinamide	1.00	1.00
D-Pantothenic Acid (hemicalcium salt)	1.00	1.00
Pyridoxal • HCl	1.00	1.00
Retinol Acetate	0.10	0.10
Riboflavin	0.10	0.10
Thiamine • HCl	1.00	1.00
DL-α-Tocopherol Phosphate • 2Na • $2H_2O$	0.01065	0.01065
Vitamin B-12	0.20	0.20
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	264.90	264.90
Cupric Sulfate • $5H_2O$	0.00009	0.00009
Ferric Nitrate • $9H_2O$	0.0001	0.0001
Magnesium Sulfate • $7H_2O$	200.00	200.00
Manganese Chloride • $4H_2O$	0.0001	0.0001
Potassium Chloride	400.00	400.00
Sodium Bicarbonate	2200.00	--
Sodium Chloride	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	140.00	140.00
Zinc Sulfate • $7H_2O$	0.0002	0.0002
OTHER		
D-Glucose	2000.00	2000.00
Glutathione (reduced)	0.05	0.05
Methyl Linoleate	0.03	0.03
Phenol Red (sodium)	10.00	10.00
Pyruvic Acid • Na	25.00	25.00

Balanced Salt Solutions

The use of balanced salt solutions in cell culture first began more than 100 years ago. Sydney Ringer, in 1885, developed an inorganic salt solution to maintain mammalian heart tissue contractility. Tyrode developed a more general salt solution for use with mammalian cells which became the standard for diluting protein components of media of natural origin. Since this pioneer work, numerous balanced salt solutions have been developed for cell culture applications. Today, balanced salt solutions serve as an irrigating, diluting and transportation fluid maintaining osmotic balance, provide cells with essential water and inorganic ions for cell metabolism, serve as a buffering source to preserve proper physiological pH balance and when supplemented with carbohydrate, like glucose, fuel cell metabolism as the principal energy source.

DULBECCO'S PHOSPHATE BUFFERD SALINE

COMPONENT	1860054 1X Liquid mg/L	1860454 1X Liquid mg/L	1861054 1X Liquid mg/L	1960054 10X Liquid mg/L
INORGANIC SALTS				
Calcium Chloride • $2H_2O$	132.50	--	132.50	1325.00
Magnesium Chloride • $6H_2O$	100.00	--	100.00	1000.00
Potassium Chloride	200.00	200.00	200.00	2000.00
Potassium Phosphate Monobasic (anhydrous)	200.00	200.00	200.00	2000.00
Sodium Chloride	8000.00	8000.00	8000.00	80000.00
Sodium Phosphate Dibasic (anhydrous)	1150.00	1150.00	1150.00	11500.00

COMPONENT	1960454 10X Liquid mg/L	1961054 10X Liquid mg/L	1760022 Powder mg/L	1760422 Powder mg/L	2810305 Tablets mg/L
INORGANIC SALTS					
Calcium Chloride • $2H_2O$	--	1325.00	132.50	--	--
Magnesium Chloride • $6H_2O$	--	1000.00	100.00	--	--
Potassium Chloride	2000.00	2000.00	200.00	200.00	200.00
Potassium Phosphate Monobasic (anhydrous)	2000.00	2000.00	200.00	200.00	200.00
Sodium Chloride	80000.00	80000.00	8000.00	8000.00	8000.00
Sodium Phosphate Dibasic (anhydrous)	11500.00	11500.00	1150.00	1150.00	1150.00

EARLE'S BALANCED SALTS

COMPONENT	1800054 1X Liquid mg/L	1800254 1X Liquid mg/L	1800454 1X Liquid mg/L
INORGANIC SALTS			
Calcium Chloride • $2H_2O$	264.90	264.90	--
Magnesium Sulfate • $7H_2O$	200.00	200.00	--
Potassium Chloride	400.00	400.00	400.00
Sodium Bicarbonate	2200.00	2200.00	2200.00
Sodium Chloride	6800.00	6800.00	6800.00
Sodium Phosphate Monobasic • H_2O	140.00	140.00	140.00
OTHER			
D-Glucose	1000.00	1000.00	1000.00
Phenol Red • Na	10.00	--	10.00

COMPONENT	1910054 10X Liquid mg/L	1700122 Powder mg/L
INORGANIC SALTS		
Calcium Chloride • $2H_2O$	2649.00	264.90
Magnesium Sulfate • $7H_2O$	2000.00	200.00
Potassium Chloride	4000.00	400.00
Sodium Bicarbonate	--	--
Sodium Chloride	68000.00	6800.00
Sodium Phosphate Monobasic • H_2O	1400.00	140.00
OTHER		
D-Glucose	10000.00	1000.00
Phenol Red • Na	100.00	10.00

HANKS' BALANCED SALTS

COMPONENT	1810054 1X Liquid mg/L	1810254 1X Liquid mg/L	1810454 1X Liquid mg/L	1810554 1X Liquid mg/L
INORGANIC SALTS				
Calcium Chloride • $2H_2O$	185.50	185.50	--	--
Magnesium Sulfate • $7H_2O$	200.00	200.00	--	--
Potassium Chloride	400.00	400.00	400.00	400.00
Potassium Phosphate Monobasic (anhydrous)	60.00	60.00	60.00	60.00
Sodium Bicarbonate	350.00	350.00	350.00	350.00
Sodium Chloride	8000.00	8000.00	8000.00	8000.00
Sodium Phosphate Dibasic (anhydrous)	47.50	47.50	47.50	47.50
OTHER				
D-Glucose	1000.00	1000.00	1000.00	1000.00
Phenol Red • Na	10.00	--	10.00	--

COMPONENT	1910154 10X Liquid mg/L	1910654 10X Liquid mg/L	1710122 Powder mg/L
INORGANIC SALTS			
Calcium Chloride • $2H_2O$	1855.00	--	185.50
Magnesium Sulfate • $7H_2O$	2000.00	--	200.00
Potassium Chloride	4000.00	4000.00	400.00
Potassium Phosphate Monobasic (anhydrous)	600.00	600.00	60.00
Sodium Bicarbonate	--	--	--
Sodium Chloride	80000.00	80000.00	8000.00
Sodium Phosphate Dibasic (anhydrous)	475.00	475.00	47.50
OTHER			
D-Glucose	10000.00	10000.00	1000.00
Phenol Red • Na	100.00	100.00	10.00

AMINO ACID CONCENTRATES

COMPONENT	1600154 BME 100X Liquid mg/L	1601149 MEM 50X Liquid mg/L	1681049 MEM NEAA 100X Liquid mg/L
AMINO ACIDS			
L-Alanine	--	--	890.00
L-Arginine • HCl	2106.00	6320.00	--
L-Asparagine • H_2O	--	--	1320.00
L-Aspartic Acid	--	--	1330.00
L-Cystine • 2Na • H_2O	1511.00	1511.00	--
L-Glutamic Acid	--	--	1470.00
Glycine	--	--	750.00
L-Histidine • HCl • H_2O	1050.00	2095.00	--
L-Isoleucine	2623.00	2625.00	--
L-Leucine	2623.00	2625.00	--
L-Lysine • HCl	3653.00	3653.00	--
L-Methionine	746.00	745.00	--
L-Phenylalanine	1651.00	1651.00	--
L-Proline	--	--	1150.00
L-Serine	--	--	1050.00
L-Threonine	2382.00	2382.00	--
L-Tryptophan	408.00	510.00	--
L-Tyrosine • 2Na • $2H_2O$	2595.00	2595.00	--
L-Valine	2343.00	2345.00	--

VITAMIN CONCENTRATES

COMPONENT	1600454 BME 100X Liquid mg/L	1601449 MEM 100X Liquid mg/L
VITAMINS		
D-Biotin	100.00	--
Choline Chloride	100.00	100.00
Folic Acid	100.00	100.00
myo-Inositol	200.00	200.00
Niacinamide	100.00	100.00
D-Pantothenic Acid (hemicalcium salt)	100.00	100.00
Pyridoxal • HCl	100.00	100.00
Riboflavin	10.00	10.00
Sodium Chloride	8500.00	8500.00
Thiamine • HCl	100.00	100.00