CELL CULTURE KITS

Aurora™ Chemiluminescent Detection Systems

- *Fast*
- *Versatile*
- *Non-Radioactive*
- *"Ultra-Sensitive"*

The Aurora™ Western Blot protein detection system and Southern Blot nucleic acid detection system provide rapid, ultra-sensitive detection of membrane-bound protein or nucleic acid without the use of radiochemicals. Both systems utilize ICN's StarLight™ chemiluminescent substrate for prolonged signal emission creating highly sensitive detection systems with short film exposures and superior band resolution.

This Aurora™ Western Blot system rivals the sensitivity of methods incorporating radioactive iodine (125I). Results are obtained with exposures in 30 seconds to 15 minutes with PVDF membranes, 5-15 minutes with nylon membranes, and 10-45 minutes with nitrocellulose membranes. The StarLight™ emission following incubation with the blot will last up to 48 hours allowing for multiple exposures to be done. The versatility of the kit permits easy stripping and reprobing, as well as, hard copy film images to be archived. Additionally, it can be used with any transfer membrane of preference.

The Aurora™ Southern Blot system can detect less than 1.0 picogram of membrane bound DNA with biotin labeled probes. For southern blots with alkaline phosphatase oligonucleotide probes, single copy gene detection is possible with as little as 0.25 μg of DNA. Results are obtained with exposures in 5-20 minutes. The StarLight™ emission following incubation will last up to 48 hours allowing for multiple exposures.

The versatility of the kit permits easy stripping of DNA probe and chemiluminescent material for subsequent reprobing. It can be used with biotin labeled DNA and RNA probes, as well as, digoxigenin, fluorescein, and alkaline phosphatase labeled probes. Chemiluminescence is initiated upon incubation of the nylon membrane with StarLight™ enabling detection of the nucleic acids on X-ray or instant film.

Aurora™ chemiluminescent detection systems eliminate the hazards and problems associated with the handling, shipping, storage, and licensing of radioactive compounds, as well as, the frequent reordering of fresh label which radioactive methods always require.

Aurora™ Western Blot Systems

Secondary Antibody	Catalog No.	Quantity
Goat, anti-rabbit IgG-AP	821538	10 blots
	821539	40 blots
Goat, anti-human IgG-AP	821540	10 blots
	821541	40 blots
Goat, anti-mouse IgG/IgM-AP	821536	10 blots
	821537	40 blots
Goat, anti-rat IgG/IgM-AP	821542	10 blots
	821543	40 blots

Aurora™ Southern Blot System

	Catalog No.	Quantity
Complete system	821559	10-20 blots
	821560	50-100 blots

Aurora™ Reporter Gene Assays

- *Fast*
- *Versatile*
- *Non-Radioactive*
- *"Ultra-Sensitive"*
- *Accurate*
- *Reproducible*
- *Easy Operation*

Reporter gene assays have become the most powerful tools as indicators of gene expression, revealing the effectiveness of gene manipulations. They allow insight into the finest gene regulations, serving as valuable indicators in gene therapy experiments. Their onset may signal toxic mechanisms like viral infections or oncogenic processes. Finally, the efficacy of therapeutic drugs and treatments may be assessed through the use of reporter gene assays[1].

Commonly used reporter genes include Chloramphenicol Acetyl Transferase (CAT) and β-Galactosidase (β-GAL). Both assays yield products which are quantified in cell extracts. Recently, Secreted Alkaline Phosphatase (SEAP) has grown as a powerful alternative[2,3]. It has the enormous advantage of utilizing a secreted protein permitting direct measurement in the culture medium without disrupting the cells. This feature allows the same culture to be monitored over a defined period of time. Furthermore, SEAP assays permit study of post-translational processes like glycosylation and secretion[4]. Another alternative currently used, β-Glucuronidase (β-GUS) serves as a popular method for determination of plant or mammalian gene expression[5].

ICN's Aurora™ reporter gene kits offer superior, sensitive quantitation of β-GAL, SEAP, and β-GUS. Likewise, they offer substantial benefits over the classical assays for these proteins which lack sensitivity and suffer from a narrow dynamic range, especially CAT[6]. The detection sensitivity is dramatically increased through the use of the StarLight™ substrate. For example, the Aurora™ Gal-XE kit for β-galactosidase exhibits greater than four orders of magnitude more sensitivity compared to colorimetric assays.

Each reporter gene assay is fast, accurate, and easy-to-perform. Adding to their convenience, their remarkably wide dynamic range eliminates meticulous serial sample dilutions. This translates into additional savings in time, labor, reagents and expense. Typically, the reaction is performed directly in a luminometer cuvette and the chemiluminescence is measured using a luminometer. Alternatively, a liquid scintillation counter may be used[7,8].

Ref.:
1. Alam, J. and Cook, J.L., Anal. Biochem., **188**, 245-254 (1990).
2. Berger, J.J., et al., Gene, **66**, 1-10 (1988).
3. Cullen, B.R. and Malim, M., Methods in Enzymology, **216**, 362-368 (1992).
4. Davis, T.R., et al., Biotechnology, **10**, 1148-1150 (1992).
5. Martin, T., et al., In S.R. Gallagher (ed.), GUS protocols: Using the GUS gene as a reporter of gene expression, 23-43 (1992).
6. Jain, V.K. and Magrath, I.T., Anal. Biochem., **199**, 119-124 (1991).
7. Fulton, R. and Van Ness, B., Biotechniques, **15(5)**, 762-763 (1993).
8. Nguyen, V.T., et al., Anal. Biochem., **171**, 404-408 (1988).

Aurora™ Gal-XE

Aurora™ Gal-XE is designed for the rapid, " ultra-sensitive " detection of β-galactosidase in cell extracts in reporter gene assays. This kit offers greater than 1,000 fold sensitivity than the color assay using o-nitrophenyl-β-D-galactoside (ONPG) and up to 1,000 fold greater sensitivity than both the isotopic and non-isotopic reporter assays for chlormaphenicol acetyl transferase (CAT). As little as 4,000 molecules can be detected. The high level of sensitivity makes this assay ideal for the detection of weak expression and for transfection normalization with other sensitive reporter assays such as luciferase. Moreover, the simplicity, sensitivity, and broad dynamic range of this assay enables rapid optimization of transfection conditions.

Item	Catalog No.	Quantity
Aurora™ Gal-XE Detection System	3121000 3122000	200 assays 600 assays
Aurora™ Gal-XE Reaction Buffer Diluent	3122005	120 ml
Aurora™ Accelerator	3142003	210 ml
Aurora™ Gal-XE Basic Vector	3121010	20 µg
Aurora™ Gal-XE Promoter Vector	3121020	20 µg
Aurora™ Gal-XE Enhancer Vector	3121030	20 µg
Aurora™ Gal-XE Control Vector	3121040	20 µg

Aurora™ AP

Aurora™ AP is designed for the quantitation of secreted alkaline phosphatase (SEAP) in culture media in reporter gene assays. Secreted reporter proteins have a major advantage since the preparation of cell extracts is not required. Furthermore, as only a small amount of medium is needed for the assay, the system allows for the monitoring of the same cell population over a period of time. With a detection limit of less than 10 femtograms, the assay is 3 orders of magnitude more sensitive than the isotopic CAT assay and the colorimetric detection for SEAP. Furthermore, it rivals the sensitivity of luciferase. As for Aurora™ AP, the wide linear range of the assay enables fast and accurate intra-assay comparisons without making several sample dilutions.

Item	Catalog No.	Quantity
Aurora™ AP Detection System	3131000 3132000	200 assays 600 assays
Aurora™ Accelerator	3142003	210 ml
Aurora™ AP Basic Vector	3131010	20 µg
Aurora™ AP Promoter Vector	3131020	20 µg
Aurora™ AP Enhancer Vector	3131030	20 µg
Aurora™ AP Control Vector	3131040	20 µg

Aurora™ Gus

Aurora™ Gus is designed for the quantitative detection of bacterial β-glucuronidase in reporter gene assays and is up to 100 times more sensitive than the fluorescent assay incorporating 4-methylumbelliferyl-β-D-glucuronide (MUG). Unlike MUG, Aurora™ GUS is not affected by fluorogenic compounds found in plant material and is not subject to disturbing quenching effects at high protein levels. The dynamic range covers 6 orders of magnitude (10^{-1} to 10^{5} picograms of β-glucuronidase) and cannot be rivaled by colorimetric or fluorescent assay methods. These characteristics, along with the rapid assay format and compatibility with luciferase lysis buffers, make Aurora™ GUS ideal for the normalization of transfection efficiencies with the luciferase reporter. Both assays are compatible with the same instrument, delivering prompt normalized data.

Item	Catalog No.	Quantity
Aurora™ Gus Detection System for plant cells	3141000 3142000	200 assays 600 assays
Aurora™ Gus Detection System for mammalian and other animal cells	3143000 3144000	200 assays 600 assays
Aurora™ Gus Reaction Buffer Diluent	3142005	120 ml
Aurora™ Accelerator	3142003	210 ml

Expression and Cloning Vectors

0921100 -20°C **AcMNPV C6 Wild Type Virus** — 2 ml

0910400 -20°C **pAcUW31 TRANSFER VECTOR** — 15 µg
Transfer vector for high-level expression of cloned genes under the control of **two** potent AcMNPV polyhedrin promoters. Designed for high level expression of two different genes in the same cell.
Complete construct information is available upon request.

0910100 -20°C **BAC-UP6 VIRAL DNA** — 15 µg
Derivative of AcMNPV that facilitates the production of recombinant viral expression vectors from AcMNPV transfer vectors.
A transfection reagent is provided with Bac-Up6 DNA for efficient co-transfections.

0910200 -20°C **pBAC-UP8** — 15 µg
Transfer vector for high-level expression of cloned genes under the control of the potent AcMNPV polyhedrin promoter.
Complete construct information is available upon request.

0910300 -20°C **pBAC-UP9** — 15 µg
Transfer vector for high-level expression of cloned genes under the control of the potent AcMNPV polyhedrin promoter.
Complete construct information is available upon request.

0920200 -20°C **BAC-UP BACULOVIRUS EXPRESSION KIT** — 1 kit
The complete kit includes the following material, sufficient to perform 5 transfections:
- 2 Different transfer vectors for high-level expression of cloned genes driven by the potent AcMNPV polyhedrin promoter.
- Bsu36 I viral DNA digest.
- Transfection reagent.
- IPLB-Sf21 *Spodoptera frugiperda* cells.
- Positive control virus stock.
- Negative control AcMNPV wild-type virus.
- Bac1 sequencing/PCR Primer.
- Bac2 sequencing/PCR Primer.
- Positive control plasmid pBac-up8-GUS.

Biogenic Amine ELISA Kits

Biogenic amines are significantly involved in the regulation of bodily functions, signal transduction processes, neurotransmission, cell to cell interactions and hormone secretion. Because of their relationship to these processes, biogenic amines have become increasingly important in neuroscience research. However, their quantification in biological systems and fluids have been historically difficult, inconvenient and expensive requiring sophisticated methods involving HPLC and GC/MS.

Today, ICN offers convenient, easy-operation immunoassays for routine determination of biogenic amines such as metanephrines, histamine, serotonin, melatonin and 5-HIAA in serum and urine samples. All assays demonstrate excellent correlation to HPLC and GC/MS methods. The assays follow the basic principles of competitive ELISA and includes a common affinity extraction in macrotiter plates and subsequent enzymatic methylation and chemical N-acylation. After an overnight incubation, followed by the addition of either AP or HRP labelled antibody, the substrate reaction can then be measured.

FOR RESEARCH PURPOSES ONLY.

Biogenic Amine Kits

ELISA	Cat. No.	Standard Range	Quantity
Adrenaline	193589	1.4-150 ng/ml	1 kit
sCD14	193599	5.5-90 ng/ml	1 kit
5-HIAA	193592	0.4-55.0 mg/L	1 kit
Histamine	193590	0.67-162 ng/ml	1 kit
Histamine Release (not ELISA format)	193591	n/a	1 kit
Melatonin	193596	3-300 pg/ml	1 kit
Metanephrine	193593	26-2500 µg/L	1 kit
Noradrenaline	193594	5-500 ng/ml	1 kit
Normetanephrine	193595	77-7500 µg/L	1 kit
Serotonin	193598	0.05-11 ng/ml	1 kit
6-Sulfatoxymelatonin	193597	1.7-420 ng/ml	1 kit

Cytokine ELISA Kits

ELISA-Kine Plus™ - TOTAL determination of cytokine.

- Sensitivity - detects up to nanogram quantities of cytokine for enhanced clinical correlation which conventional assays overlook.
- Flexibility - optimized for various sample types including serum, plasma, slaiva, cell culture supernatant and other biological fluids.
- Simplicity - a standard, uniform format with a 3 hour incubation period across the entire product line.
- Specificity - tested for cross-reactivity against WHO cytokine standards. No detectable cross-reactivity between cytokines.
- Productivity - 192 determinations in standard 96 well microplate format, twice as many as traditional assays.
- Validity - assured reliability of detection by parallelism and quantitative recovery studies.
- Predictive Relevancy - accurate measurement of LPS and superantigen cytokine induction.
- Compatibility - may be used in correlation with conventional assys for "non-bound" versus "bound" cytokine comparison in a particular sample. Also, it may be used in place of conventional assays.

ICN's ELISA-Kine Plus™ is a unique ELISA system that measures the "Total", bound and non-bound, cytokine levels in serum, plasma, CSF, saliva and other biological fluids. Traditional cytokine ELISA assays miss the presence of all cytokine in most samples because of binding to soluble receptors, auto-antibodies and binding proteins. The ELISA-Kine Plus™ system is not hindered by these interfering factors and offers enhanced detection of true levels of cytokine in nanogram quantities. Additionally, this system will accurately measure cytokine concentration present in cell cultures providing the concentration is within the dynamic range of the assay (an alternative assay with high sensitivity in the picogram range is ICN's ELISA-Kine™ direct cytokine assay system).

ELISA-Kine Plus™ is a polyclonal based "competitive" enzyme immunoassay (EIA) which effectively measures both natural and recombinant forms of cytokines. With this system, pre-coated, goat anti-rabbit antibodies are used to capture a specific protein complex in each well that contains the polyclonal cytokine antibody, biotinylated cytokine and sample/standard. The use of a polyclonal antibody capture results in superior detection of the entire population of cytokine as compared to epitope-specific monoclonals.

In summary, the cytokine specific antibody, biotinylated cytokine conjugate (competitive ligand), and sample cytokine form a competition reaction in which samples compete for antibody binding sites with the biotinylated cytokine. As the concentration of the sample cytokine increases, the amount of biotinylated cytokine captured by the antibody decreases. Upon addition of the streptavidin-alkaline phosphatase conjugate, substrate solution and amplifier solution, the amount of biotinylated cytokine is detected. The result is an inverse relationship between Optical Density (OD) and concentration: the greater the OD, the less sample cytokine is present.

ELISA-Kine Plus™ kits are available for human and mouse systems. All kits are supplied with enough reagents for 2 x 96 well determinations. Results are produced in four hours and the shelf-life is 6 months when properly stored at 4°C. ICN recommends running duplicate wells for samples and standards. In addition, analysis of serum or plasma samples requires they be diluted at least 1:2 with Assay Diluent (i.e. 50 µl of sample and 50 µl of Assay Diluent per well), and the standards should be diluted in Serum Diluent. Cell culture samples may be run undiluted, with standards diluted in culture media.

ELISA-Kine Plus™ Kits - Human

Cytokine Assay	Catalog No.	Quantity
IL-1α	3230000	1 kit
IL-1β	3230200	1 kit
IL-2	3230300	1 kit
IL-3	3230400	1 kit
IL-4	3230500	1 kit
IL-6	3230600	1 kit
IL-7	3230700	1 kit
IL-8	3230800	1 kit
IL-10	3230900	1 kit
IL-13	3231000	1 kit
GM-CSF	3231100	1 kit
TNF-α	3231200	1 kit
TNF-β	3231300	1 kit
IFN-α	3231400	1 kit
MIP-1α	3231500	1 kit
VEGF	3231600	1 kit
FGF-b	3231700	1 kit
EGF	3231800	1 kit

ELISA-Kine™ Kits - Human

Cytokine Assay	Catalog No.	Quantity	Price
IL-1α	3233000	1 kit	
IL-1β	3233100	1 kit	
IL-2	3233200	1 kit	
IL-4	3233300	1 kit	
IL-5	3233400	1 kit	
IL-6	3233500	1 kit	
IL-10	3233600	1 kit	
IL-15	3234100	1 kit	
GM-CSF	3233700	1 kit	
TNF-α	3233800	1 kit	
IFN-γ	3233900	1 kit	
Rantes	3234000	1 kit	

ELISA-Kine Plus™ Kits - Mouse

Cytokine Assay	Catalog No.	Quantity
IL-1α	3230100	1 kit
IL-1β	3231900	1 kit
IL-3	3232000	1 kit
IL-6	3232100	1 kit
GM-CSF	3232200	1 kit
TNF-α	3232300	1 kit

ELISA-Kine™ Direct Assay Kits

- Sensitive - detection level often lower than 1 pg/ml.
- Econcomical - standard 192 (2 x 96 well) determinations.
- Flexible - ideal for cell culture, serum, plasma and other fluids.
- Efficient - easy-to-use standard protocol and 3 hour incubation.
- Versatile - compare to ELISA-Kine Plus™ for complete determination.

ELISA-Kine™ kits offer sensitive detection of cytokine levels in cell culture and other biological fluids. The system follows conventional methods by employing a monoclonal/polyclonal based sandwich enzyme immunoassay (EIA) protocol. The ELISA-Kine™ system effectively detects and measures both natural and recombinant cytokine forms which are "non-bound" in a sample. To ensure superior amplification of the signal for maximum sensitivity, the ELISA-Kine™ system uses a two-step color generating procedure. Alkaline phosphatase dephosphorylates NADPH to NADH, which then serves as the cofactor which activates a cycling redox reaction driven by alcohol dehydrogenase and diaphorase.

Each ELISA-Kine™ kit follows a standard procedure, thereby eliminating any need to become familiar with multiple protocols. Murine monoclonal antibodies, that have been generated against specific cytokines, capture protein from the sample in a 3 hour incubation. Next, rabbit anti-cytokine antibodies detect the captured cytokine. The addition of the goat anti-rabbit-alkaline phosphatase conjugate, followed by substrate and amplifier, provides the basis for the highly consistent, reproducible and sensitive detection system. The presence of protein is determined through a rapidly developing deep red colored product (formazan) which absorbs light at 492 nm. The resulting curve demonstrates a direct relationship between the Optical Density (OD) and cytokine concentration throughout the entire dynamic range.

Endotoxin Detection Kit

3070000 0-5°C — **ENDOTOXIN DETECTION KIT** — 20 tests
Based on Limulus Amebocyte Lysate (LAL) assay
- Excellent Reproducibility
- Simple-to-Use
- Clear Determination- (+) or (-)
- Economical
- Sensitive

The Endotoxin Detection Kit is a complete 20 test kit. Sensitivity level: 0.06 ng/ml. The contents include:
22 Single Test Vials Lysate
5 Inhibition Control Vials
1 Positive Control Vial
1 LAL Reagent Water (<0.005 EU/ml)
28 Pipettes
1 Sheet of Labels.

3070200 0-5°C — **ENDOTOXIN DETECTION KIT** — 20 tests
Based on Limulus Amebocyte Lysate (LAL) assay
- Excellent Reproducibility
- Simple-to-Use
- Clear Determination- (+) or (-)
- Economical
- Sensitive

The Endotoxin Detection Kit is a complete 20 test kit. Sensitivity level: 0.125 ng/ml. The contents include:
22 Single Test Vials Lysate
5 Inhibition Control Vials
1 Positive Control Vial
1 LAL Reagent Water (<0.005 EU/ml)
28 Pipettes
1 Sheet of Labels.

3070100 0-5°C — **ENDOTOXIN CONTROL KIT** — 1 kit
Additional controls for use with the Endotoxin Detection Kit (cat. no. 30-700-00). The kit contains:
5 Positive Control Vials
5 Negative Control Vials.

Mycoplasma Stain Kit

- Reliable: Uses Hoechst Stain Method.
- Complete Includes Stain, Diluent, Mounting Medium and Controls.
- Versatile: Detects Mycoplasma, Bacteria, Yeast and Fungi.
- Rapid: Requires Less Than 2 Hours.
- Economical: 100 Tests/Kit.

The ICN Mycoplasma Stain Kit is ideal for the *in situ* detection of mycoplasma and other prokaryotic organisms in cell cultures. This kit employs the Hoechst stain method cited by the Tissue Culture Association (TCA procedure no. 75361). It contains Hoechst stain 33258 (*bis*-Benzimide), a widely used fluorescent stain for chromosomes and DNA due to the ability of *bis*Benzimide to intercalate DNA.

DNA fluorochrome staining is both rapid and sensitive. A cell sheet that is 50-80% confluent is fixed and stained with a DNA specific dye and examined via fluorescent microscopy. Non-nuclear staining will be readily apparent and contaminants "stand-out" boldly against a black background. The nature of the contaminant may be determined by its morphology, size, and relationship to the cells. The Hoechst stain method performs better than other fluorochromes in that a more pronounced fluorescent effect with minimal background and quenching results.

Kit Contents:

1x10 ml Hoechst Stain 33258
3x35 ml 1X HBSS without phenol red and sodium bicarbonate
1x10 ml Mounting Medium
10 Positive fixed control slides - 3T-6 fibroblasts infected with *M. hyorhinis* (DDS-1050)
10 Negative fixed control slides - 3T-6 non-infected fibroblasts
1 Protocol booklet

For a copy of the protocol, please contact ICN Customer Service.

Ref.: 1. Chen, Experimental Cell Research, **104**, 255-262 (1977).

	Catalog No.	Quantity
Mycoplasma Stain Kit	3030000	100 Tests

ImmuMark™ MycoTest™

For Rapid Detection of Mycoplasma Using Fluorescent Antibodies!

- Direct Testing of Cell Suspensions
- Highly Specific
- Fluorescence Can Be Amplified
- Higher Resolution Than Other Methods

Mycoplasma infection in cell cultures is generally a chronic infection which may not be obvious by visual inspection or light microscopy. As such, routine and periodic screening of cell cultures for mycoplasma is important. A variety of tests for the detection of mycoplasma are currently available such as fluorochrome staining of DNA, monitoring toxic metabolites, culture methods, etc., but each method has certain disadvantages. The primary drawbacks to these methods are a lack of specificity and time-consuming protocols.

The ICN ImmuMark™ MycoTest™ employs the monoclonal antibody CCM-2 specific for a broad range of mycoplasma species such as *Acholeplasma laidlawii, Mycoplasma hyorhinis, Mycoplasma arginini, Mycoplasma orale,* and *Mycoplasma salivarium*, which account for more than 96% of all cell culture infections. The test combines monoclonal antibody specificity with very short assay times. The fluorochrome labeled antibody which is included allows for very sensitive detection of mycoplasma.

The ImmuMark™ MycoTest™ offers two methods of detection:

- One Step Assay - Suspected contaminated samples are directly screened by the fluorochrome labeled monoclonal antibody CCM-2.
- Two Step Assay- Suspected contaminated samples are indirectly screened using the CCM-2 fluorochrome labeled antibody and a fluorochrome labeled secondary antibody for extremely sensitive detection.

For both methods, specimens are incubated for 20 minutes with one or both conjugates. Excess reagent is washed off with PBS. The mounted slides are viewed microscopically using epifluorescent illumination. If mycoplasma contamination exists, a characteristic yellow-green fluorescence is seen on cell perimeters and between counter-stained infected cells which will appear bright red.

Kit Contents:

2 ml Monoclonal CCM-2 Fluos conjugate, diluted in protein stabilized buffer with Evans blue counterstain.
2 ml Goat, Anti-Mouse IgG FITC conjugate, diluted in protein stabilized buffer with Evans blue counterstain.
2.5 ml Mounting medium.
1 Protocol booklet.

	Catalog No.	Quantity
ImmuMark™ MycoTest™	3020000	50 Tests