

# Labelling techniques

This issue covers the reagents and equipment necessary for cellular and molecular imaging and detection, including a luminometer, a phosphor imaging system, reagents to detect intracellular cytokines and alternatives to radiolabelling.

VAYTEK has developed CoLocalization for those who use multiple fluorescent probes in capturing a separate image or images for each probe colour. This new software module should allow researchers to determine the amount of overlap between the images. Quantitative measurements are said to be easy to obtain with this module. For example, the percentage of overlap and the probability that a pattern is greater than chance can be determined. The system requires a Pentium computer running either Windows 3.1 or Windows95 with at least 16 MB of RAM. VayTek's modular approach should make it easy to retrofit, customize and upgrade image analysis systems.

Reader Enquiry No. 100

## Reagents and kits

A new non-radioactive ELISA that is designed to provide an accurate measurement of true cellular proliferation is now available from Amersham. Using a highly specific anti-5-bromo-2'-deoxyuridine (BrdU) conjugate, the Biotrak cell proliferation ELISA offers stated detection levels of 100 cells per well in a microtitreplate format. The ELISA should be useful



**Biotrak cell proliferation ELISA.**

for studying a variety of areas, including the effects of growth factors and cytokines; the determination of inhibitory or stimulatory effects of compounds in environmental, biomedical and pharmaceutical research; and the determination of the chemosensitivity of tumour cells to different cytostatic drugs. Depending on the cell culture, the ELISA can be completed in just over two hours. Supplied as a fully functional test kit, the ELISA includes sufficient material to measure ten 96-well plates.

Reader Enquiry No. 101

R&D Systems now offers reagents to detect intracellular cytokines. These monoclonal antibodies are now available for the detection of intracytoplasmic forms of cytokine by flow cytometry, conjugated to phycoerythrin or fluorescein where necessary. Once the cells of interest have been fixed and permeabilized, staining reveals how cellular activation can result in cytokine synthesis. The reagents are particularly useful for studying cytokine production by T-cell subsets, thereby allowing determination of whether a type-1 or type-2 response has been elicited. Antibodies are available for the detection of intracellular IL-2, IL-4, IL6, TNF- $\alpha$  and IFN- $\gamma$ . In addition, conjugated negative controls are available for the verification of results. The ability to investigate cytokine expression at the single-cell level, by combining multiparameter analysis with the speed of flow cytometry, is likely to offer new insights into immunity.

Reader Enquiry No. 102

Boehringer Mannheim has tackled the problems associated with traditional radioactive ligand labelling methods (safety, disposal and expensive reagents) with the launch of the receptor binding analysis kit. The kit utilizes a high-efficiency labelling technique to isolate ligand-receptor complexes. It uses a new series of tetrafluorophenyl azide reagents (Atf) to eliminate the safety and environmental concerns posed by radiochemical methods and reduce the amount of time spent on training, clean-up and the administration associated with radioisotope usage. The kit is designed for convenience, with ready-to-use reagents for one-step ligand-receptor binding. The complete kit features a labelling reagent for ligands containing the Atf photoreactive residue, an active ester and a biotin label. The trifunctional labelling reagent reacts with the ligand and results in a photoactivated ligand. When activated by ultraviolet light, the photoactivated ligand is then covalently linked to the binding region of its specific receptor. The kit also contains immobilized streptavidin, which acts as a purification matrix for the isolation of the photoaffinity biotin-labelled, ligand-receptor complexes. Applications for the receptor binding analysis kit include the study of ligand-receptor, antigen-antibody and interleukin interactions.

Reader Enquiry No. 103

Sigma Immunochemicals has introduced secondary antibodies and avidin reagents conjugated to Cy3 for use in immunohistochemistry and immunocytochemistry. The available antibodies labelled with Cy3 are goat anti-human IgG, goat anti-human IgM, rabbit anti-goat IgG, sheep anti-mouse IgG and sheep anti-rabbit IgG. The avidin reagents available are ExtrAvidin and streptavidin.

Cy3 is a hydrophilic, red fluorescent dye and represents a new generation of fluorophores that are said to cause less nonspecific binding and aggregation. It is more photostable and quenching is minimized even at high

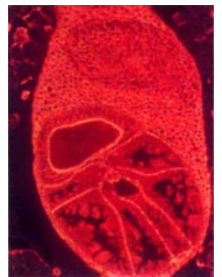
F/P ratios. Maximally excited at 554 nm, Cy3 emits at 568-574 nm and can be used with traditional TRITC filter arrangements. The Cy3-labelled avidin reagents should prove useful for detecting biotin-labelled probes by fluorescence *in situ* hybridization. Monoclonal anti-smooth muscle actin (clone 1A4) is also available labelled with Cy3.

Reader Enquiry No. 104

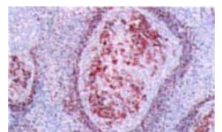
The Dako CSA system HRP is a sensitive immunohistochemical staining procedure for monoclonal antibodies, which utilizes an enzyme-catalysed deposition of biotin, resulting in an amplified signal that allows the detection of small quantities of reactive antigen. The system enables certain monoclonal antibodies, which are normally not compatible with formalin-fixed, paraffin-embedded sections, to be used on such specimens. The company is also developing a line of prediluted antibodies for use with the CSA system, which includes the option of a ready-to-use antibody.

Reader Enquiry No. 105

KPL's fluorescein, Texas-red or phycoerythrin labelled streptavidin offers high sensitivity and resolution in fluorescence microscopy. Texas-red and phycoerythrin-



**Cy3 red, hydrophilic fluorescent label.**



**Dako CSA system.**

labelled streptavidin can be used in single-colour analysis or in combination with other fluorophores for multiple labelling in flow cytometry and image analysis. AP- and HRP-labelled streptavidin are also offered. They are provided as liquid concentrates that are said to be stable for a minimum of one year.

**Reader Enquiry No. 106**

**Equipment**

Denley's Aquarius **microplate washer** incorporates an integral orbital shaker with the shaking speed automatically optimized according to the dispensed company volume. All washing cycles are user programmable through a simple four-key menu. A total of 99 wash programs can be named and stored, each having up to four linked protocols for more flexibility. Single or twin manifolds are available in 8- or 12-well formats. Moreover, the new bottle-management system allows multiple buffer usage with two 2-litre wash, the one 1-litre rinse and one 4.5-litre waste bottles, which can be switched electronically, with the 4.5-litre bottles utilizing liquid-level sensors. The software is designed to be easy to program and can handle complex programs containing up to four distinct washing protocols. For added security, the washer can be controlled from a PC and the wash procedure logged after each plate, if required.

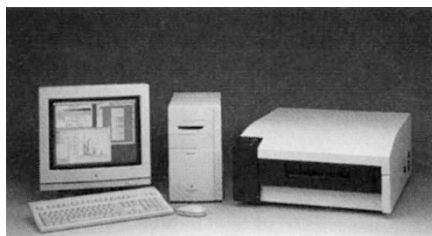
**Reader Enquiry No. 107**

The new 20/20 research **luminometer** from Steptech is designed to offer improved sensitivity and versatility. It can measure light output in its specially designed sample compartment, which accommodates an 8 mm × 50-mm test tube, a 1.5-ml microfuge tube, a 28-mm scintillation vial and a 35-mm culture dish, as well as solids of various shapes and sizes. The instrument also accommodates wavelength-specific filters and a range extender. It is sensitive to a stated 0.1 femtograms of luciferase with a wide dynamic range of 10<sup>5</sup> or greater. This can be extended to 10<sup>7</sup> or greater with a range extending optical filter. In addition, an internal automatic injector is offered as an option for routine laboratory work, allowing the system to inject variable amounts of reagents (from 50–250 µl). The ASCII output can be transmitted through the instrument's RS232 port to any PC and it will interface with Epson-compatible printers. It also has a small footprint, measuring just 23.5 cm × 28 cm × 21 cm (L × W × H).

**Reader Enquiry No. 108**

BioRad has introduced a new small format **storage phosphor imager** with true chemiluminescence and radioisotope detection for quantification on a single phosphor imaging system. The GS-505 should provide an alternative to autoradiography as it is capable of detecting

all samples suitable for storage phosphor autoradiography, including chemiluminescent samples, without needing to alter the protocols. The system accepts standard small-format (20 × 25 cm) screens, bands and regions. When these lie close together they can be easily resolved on the GS-505 due to the instrument's high spatial resolution. The imager can qualify signals for samples over a five order-of-magnitude range. Available for use with



**The GS-505 phosphor imaging system.**

Power Macintosh, Macintosh and Windows 3.1/Windows95 platforms, the system is accessible to multiple users working on different platforms. The company offers a full range of compatible hardware, which can be combined with the system. This is designed to provide inexpensive documentation, visualization and quantification from a wide variety of fluorescent stains, including ethidium bromide, SYBR Green I/II, and SYPRO Orange. The GS-505 can also be upgraded easily to a GS-525, accepting both large and small screens.

**Reader Enquiry No. 109**

The InstantImager **electronic autoradiography system** from Packard Instruments is designed to be a faster way to retrieve quantitative images, achieving higher speeds through the use of Intel Pentium machines. The system comes complete with a customized computer, enabling the instrument to display and quantitate images in real time. The high-speed, digital-signal processing now takes place within a Pentium-based system, which includes 16 MB of RAM, InstantQuant for Windows and a gigabyte of hard drive space for archiving as many as 2,500 images.

**Reader Enquiry No. 110**

**Literature**

A new **diagnostic reagents directory** is now available from the Centre for Applied Microbiology and Research (CAMR). The directory provides guidance to the organization's reagents, services and expertise. Reagents in the directory include a range of antibodies and antigens developed as a result of CAMR's research into infectious diseases and vaccine development. There are three complementary aspects of the antibody range: stock antibodies, antibodies-to-order and bulk antibodies. Facilities are

also available for the production of bulk antibodies in quantities up to 20 g from user-supplied hybridomas. CAMR also has facilities with the highest levels of containment required to grow a wide range of bacterial and viral agents, should specialized antigens be required.

**Reader Enquiry No. 111**

The 1996–1997 Immunotech **immunoassay catalogue** features cytokine, retrovirology and histamine immunoassays. The catalogue introduces several new cytokine assays, including IL-7, IL-8, IL-10, GM-CSF and sGM-CSF receptor. Several other immunoassays are also highlighted, including cyclic AMP, cyclic GMP, neopterin, protein kinase and serotonin. The catalogue details each assay providing information on reagents, procedure, standard range, sensitivity, specificity and shelf life.

**Reader Enquiry No. 112**

*These notes are compiled by Brendan Horton from information provided by the manufacturers. For more details, fill in the reader service card bound inside the journal.*

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