BIOPROBES 55

CELL BIOLOGY BY INVITROGEN

MARCH 2008

Click chemistry

Making biodiscovery a snap

Potassium channel flux assay meets BacMam technology

PLUS: Qdot® primary antibody conjugates

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Published by Invitrogen
Eugene, Oregon USA © 2008

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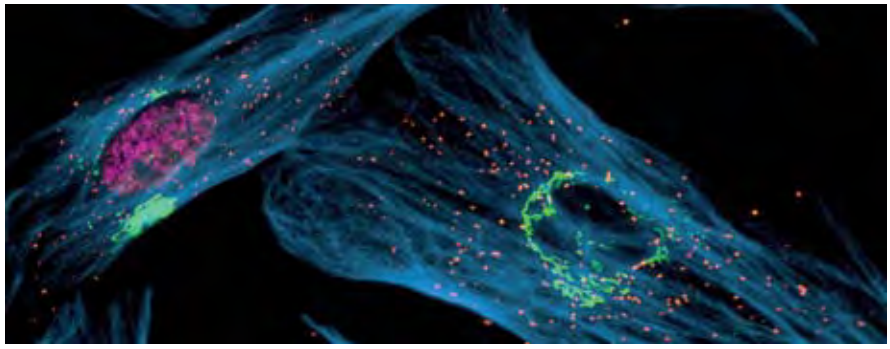

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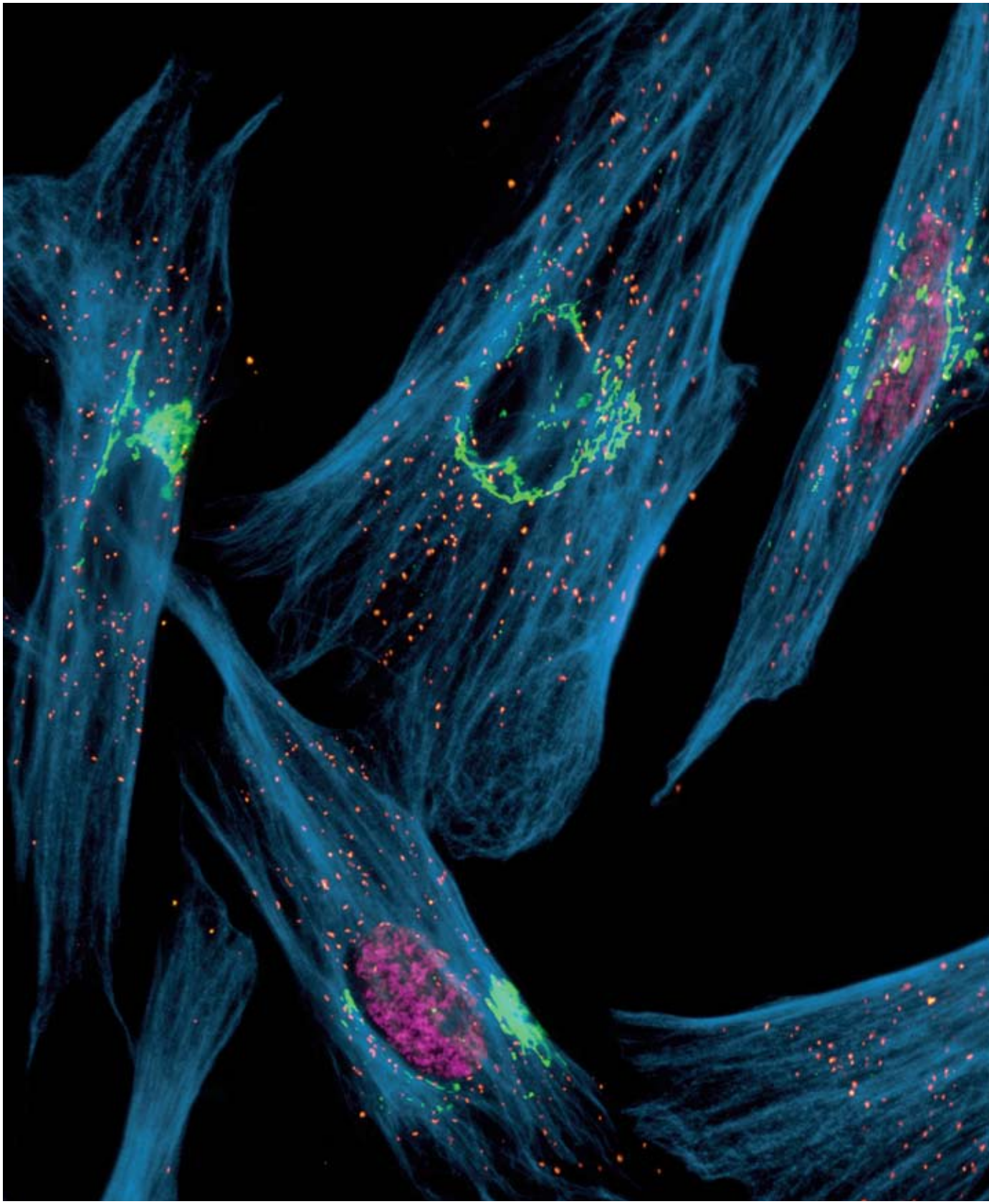
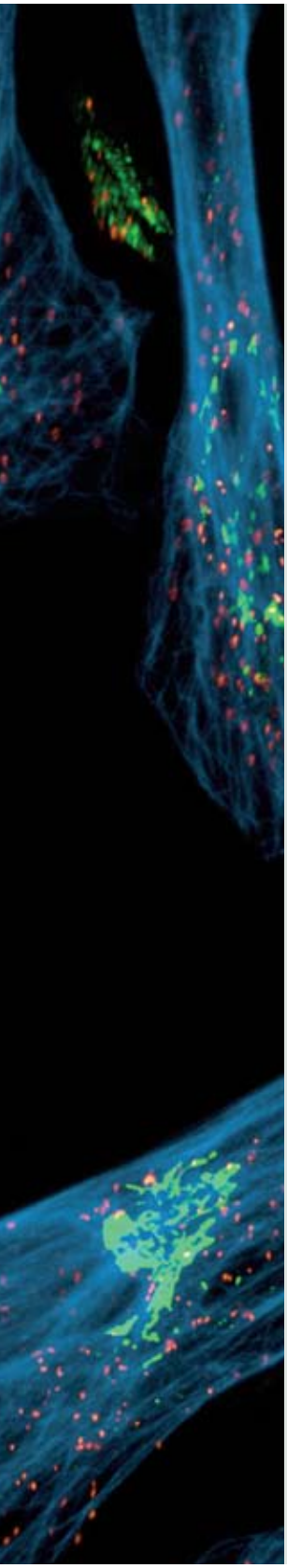
Departments





Click chemistry: Making biodiscovery a snap

REPLACE CUMBERSOME BrdU ASSAYS WITH THE SIMPLICITY OF EdU.

Whether your preferred cellular detection method is immunofluorescence or direct fluorescent labeling, what you really need are all of the advantages and none of the drawbacks. Instead, researchers often find themselves trading one set of challenges for another. The traditional detection method employs antibodies, which provide highly specific labeling; however, for an antibody to gain access to the target antigen, long incubations or harsh treatments are often required. Direct fluorescent detection, another common method, offers flexibility and ease of use. But when the label is larger than the molecule you want to visualize, the function of that molecule can be compromised. "Click" chemistry offers the best of both worlds. This unique technology provides a means of direct fluorescent labeling and detection without the background that compromises conventional amine- and thiol-reactive chemistries. Click-iT™ EdU assays offer all of the advantages of this powerful technology, providing a fast, easy, and accurate method for detecting cell proliferation (Figure 1).

Figure 1—Multicolor imaging is a snap with Click-iT™ EdU. Muntjac cells were treated with 10 µM EdU for 45 minutes. Cells were then fixed and permeabilized, and EdU that had been incorporated into newly synthesized DNA was detected using the far red–fluorescent Click-iT™ EdU Alexa Fluor® 647 Imaging Kit (Cat no. C10085). Tubulin was labeled with a mouse anti-tubulin antibody and visualized with an Alexa Fluor® 350 goat anti–mouse IgG antibody (Cat. no. A11045). The Golgi complex was stained with the green-fluorescent Alexa Fluor® 488 conjugate of lectin HPA from *Helix pomatia* (edible snail) (Cat. no. L11271), and peroxisomes were labeled with a rabbit anti-peroxisome antibody and visualized with an orange-fluorescent Alexa Fluor® 555 donkey anti–rabbit IgG antibody (Cat. no. A31572).

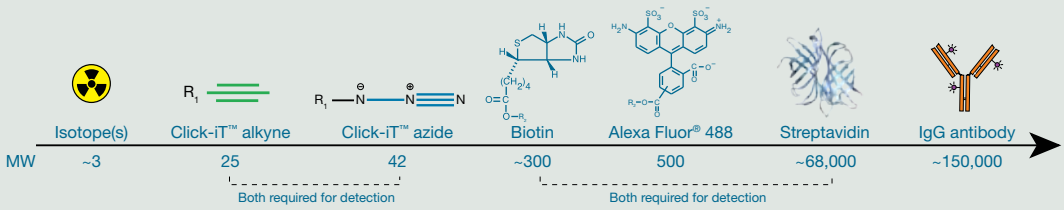


Figure 2—Relative size of detection molecules commonly used in cellular analysis. Because the azide and alkyne moieties can be used interchangeably to optimize labeling configurations, R_1 can be either the biomolecule of interest or the detection reagent. For biotin and the fluorophore, R_2 is the biomolecule of interest.

Click chemistry describes a class of chemical reactions that use bio-orthogonal or biologically unique moieties to label and eventually detect a molecule of interest using a two-step procedure. Click reactions have several characteristics: the reaction between the detection moieties is efficient; no extreme temperatures or solvents are required; the reaction product is stable; the components of the reaction are bioinert; and perhaps most importantly, no side reactions occur—the label and detection tags react selectively and specifically with one another.[1–4] This final point is the greatest advantage of this powerful detection technique; click chemistry–labeled molecules can be applied to complex biological samples and detected with unprecedented sensitivity, thanks to extremely low background.

Small size translates to big advantages

The Click-iT™ EdU cell proliferation assays are based on a two-step click reaction involving the copper-catalyzed triazole formation of an azide and an alkyne. Both azides and alkynes are biologically unique, inert, stable—and extremely small (Figure 2). These moieties can be used interchangeably; either one can be used to tag the molecule of interest while the other is used for subsequent detection. The label is small enough that tagged molecules (e.g., nucleotides, sugars, and amino acids) are acceptable substrates for the enzymes that assemble these building blocks into biopolymers.

Click chemistry fills the void when methods such as direct labeling or the use of antibodies fail or fall short. For example, depending on the size of the molecule of interest, a direct bioconjugate with biotin or a fluorophore may adversely affect the biological function of that molecule. And antibodies and other indirect detection methods may be too large to access the antigen of interest in tissues or cells without resorting to harsh permeabilization or long incubations. With its small "footprint", the Click-iT™ detection molecule can easily penetrate complex samples, including intact (supercoiled) DNA, with only mild permeabilization required.

Click-iT™ EdU—fast, easy, and accurate detection of cell proliferation

Click-iT™ EdU cell proliferation assays are top of the class for direct and accurate detection of new DNA synthesis. These assays not only measure proliferation of individual cells, but also can detect proliferating cells on virtually any platform (Table 1). Click-iT™ assays use a modified nucleoside, EdU (5-ethynyl-2´-deoxyuridine), that is incorporated during DNA synthesis. Unlike assays using bromodeoxyuridine (BrdU), Click-iT™ EdU assays are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside (Figure 3).

Table 1—Click-iT™ EdU assays.

Platform	Amount of EdU included	Number of samples	Available fluorophores *	Notes
Flow cytometry	10 mg	50 assays based upon a 0.5 ml volume	Alexa Fluor® 488 Alexa Fluor® 647 Pacific Blue™ dye	• Includes two cell cycle dyes compatible with detection fluorophore • Not interchangeable with imaging assays
High-throughput imaging (HCS)	0.255 mg (2-plate) 1.28 mg (10-plate)	2 × 96 tests (2-plate) 10 × 96 tests (10-plate)	Alexa Fluor® 488 Alexa Fluor® 594 Alexa Fluor® 647	• Includes Hoechst 33342 for cell registration or cell cycle analyses • Not interchangeable with flow cytometry assay
Fluorescence microscopy	5 mg	50 coverslips	Alexa Fluor® 488 Alexa Fluor® 594 Alexa Fluor® 647	• Includes blue-fluorescent nuclear counterstain, Hoechst 33342 • Not interchangeable with flow cytometry assay

* Each kit contains one fluorophore. See product list for kit catalog numbers.

For BrdU detection, DNA is denatured with DNase, heat, or HCl; these treatments can destroy antigen recognition sites or make it difficult to perform simultaneous cell cycle analyses on the same sample, as many dyes for cell cycle analysis require dsDNA (Table 2, Figure 4). Click-iT™ assays avoid the harsh treatments required by BrdU assays, providing a method that is more reliable and easier to perform. And although BrdU assays performed in tissue samples involve long incubations—from several hours to overnight—Click-iT™ EdU detection reactions are complete in less than 80 minutes, and often in under 30 minutes.

Cell walls are no barrier for Click-iT™ EdU

Cell walls typically provide a barrier to detecting cell proliferation in plant cells. Using antibodies not only requires DNA denaturation, but also requires that the cell wall is digested. However, cell wall–digesting enzymes often contain impurities that can decrease the reliability of the assay. Furthermore, the additional steps required can make BrdU assays labor intensive and time consuming. But even in plant cells, Click-iT™ EdU assays involve only a mild fixation and permeabilization step—no DNA denaturation or cell wall digestion is required (Figure 5). Click-iT™ EdU assays are faster, more accurate, and extremely sensitive and reliable, even on tough samples.

Multiplexed analyses are a snap with Click-iT™ EdU

Click-iT™ EdU assays are ideal for multiplexed analysis in both cells and tissues. Because only a very mild fixation and permeabilization step is required for entry of the detection moiety, not only is dsDNA retained, but antigen recognition sites are preserved. Although we recommend starting with an amount of EdU equal to the amount of BrdU normally used, researchers can often use less EdU or reduce the incubation time and still obtain the same or even better signal than that provided by the BrdU assay. After EdU is incorporated, simply fix and permeabilize, then perform the Click-iT™ detection →

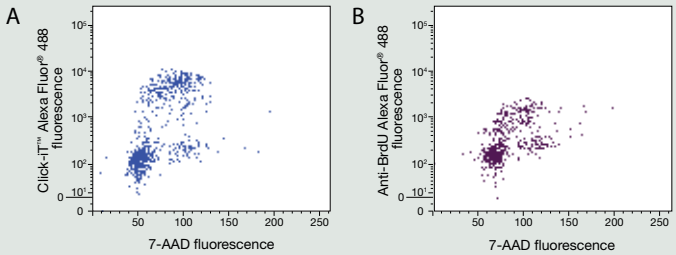


Figure 4—Results obtained using the Click-iT™ EdU reagents typically surpass those from the BrdU assay. (A) Results obtained using the new Click-iT™ EdU detection method, showing a dual-parameter plot of Click-iT™ Alexa Fluor® 488 azide vs. 7-AAD cell cycle staining. **(B)** Results using the standard acid denaturation method for the antibody-based detection of incorporated BrdU, showing a dual-parameter plot of anti-BrdU Alexa Fluor® 488 vs. 7-AAD cell cycle staining.

Table 2—Properties of several cell cycle stains compatible with Click-iT™ EdU.

Dye	Characteristics
DAPI	AT-selective
Hoechst 33342	AT-selective
Propidium iodide	Intercalator; binds both DNA & RNA; RNase recommended
7-AAD	GC-selective

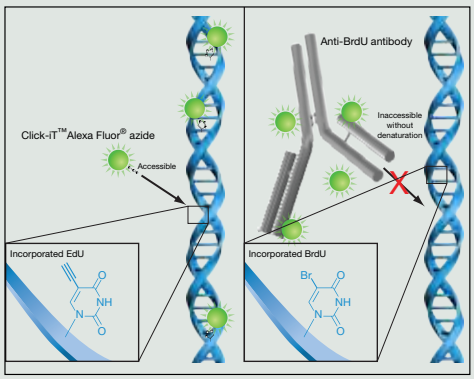


Figure 3—Detection of the incorporated EdU with the Alexa Fluor® azide versus incorporated BrdU with an anti-BrdU antibody. The small size of the Alexa Fluor® azide eliminates the need to denature the DNA in order for the detection reagent to gain access to the nucleoside.

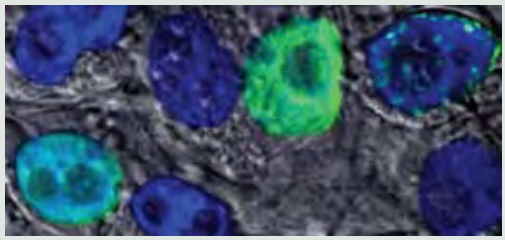


Figure 5—Cell wall digestion is not required with Click-iT™ EdU. *Medicago sativa* (alfalfa) suspension cultures were incubated with 10 µM EdU for 3 hours. Cells were then fixed and permeabilized. EdU that had been incorporated into newly synthesized DNA was detected with the Click-iT™ EdU Alexa Fluor® 488 Imaging Kit (green fluorescence, Cat. no. C10083). Nuclei were stained with blue-fluorescent DAPI. Six confocal sections were overlaid onto a differential interference contrast image. Image contributed by Ferhan Ayaydin, Cellular Imaging Laboratory, Biological Research Center, Szeged, Hungary.

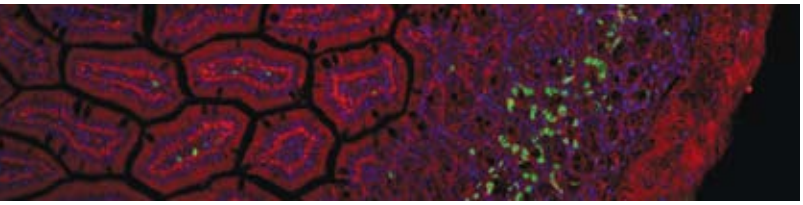


Figure 6—Proliferating cells labeled *in vivo* with the Click-iT™ EdU assay. EdU from the Click-iT™ EdU Alexa Fluor® 488 Imaging Kit (Cat. no. C10083) was administered to mice intraperitoneally 2 hours before sacrifice. After intestinal tissues were formalin fixed and paraffin embedded, EdU was labeled with the Click-iT™ reaction, using approximately 250 µl of reaction cocktail per slide. The tissue sections were then washed and treated with mounting medium containing DAPI. EdU-positive cells are labeled green, nuclei are stained blue, and red autofluorescence was enhanced for image contrast. Image contributed by Sima Zacharek, Department of Genetics, Children's Hospital Stem Cell Program, Children's Hospital Boston, Massachusetts.

reaction. Some fluorophores are not completely compatible with the copper that drives the Click-iT™ reaction, requiring adjustments to the workflow (Table 3). In most cases, antibodies, lectins, fluorescent proteins (Figures 6 and 7), or cell cycle stains can be used with Click-iT™ EdU for deeper biological insight.

Click chemistry holds enormous potential for a variety of applications. Find out what Click-iT™ EdU can do for your cell proliferation assays at **www.invitrogen.com/edu**. ■

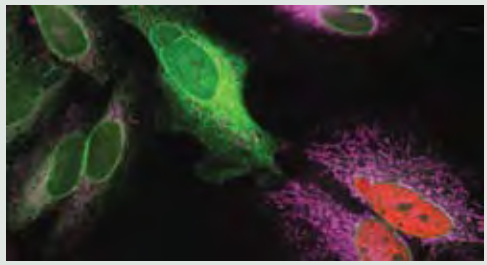


Figure 7—Click-iT™ EdU compatibility with fluorescent proteins. HeLa cells were transduced with Organelle Lights™ NE GFP (Cat. no. O36213) for 16 hours, then incubated for 60 minutes with reagents from the Click-iT™ EdU Alexa Fluor® 594 Imaging Kit (Cat. no. C10084) to detect proliferating cells (red fluorescence) and with MitoTracker® Deep Red FM (Cat. no. M22426) to detect mitochondria (pseudocolored pink). Following fixation and permeabilization, green fluorescent protein (GFP) expressed in the nuclear envelope was detected with rabbit anti-GFP serum (Cat. no. A6455) and visualized using Alexa Fluor® 488 goat anti–rabbit IgG antibody (Cat. no. A11008). Green fluorescence is also seen in the endoplasmic reticulum as it forms from the nuclear envelope.

References

1. Breinbauer, R. and Köhn, M. (2003) *ChemBioChem* 4:1147–1149.
2. Wang, Q. et al. (2003) *J Am Chem Soc* 125:3192–3193.
3. Rostovtsev, V.V. et al. (2002) *Angew Chem Int Ed Engl* 41:2596–2599.
4. Kolb, H.C. et al. (2001) *Angew Chem Int Ed Engl* 40:2004–2021.

Table 3—Click-iT™ EdU compatibility.*

Fluorescent molecules	Notes
Qdot® nanocrystals	Use Qdot® nanocrystals after the Click-iT™ detection reaction
Fluorescent proteins (GFP)	Use organic dye–labeled expression tags (e.g., TC FlAsH/ReAsH) to detect protein expression; use anti-GFP antibodies before or after the Click-iT™ detection reaction to detect and generate fluorescence †
Organic dyes (e.g., Alexa Fluor® dyes, fluorescein (FITC))	Completely compatible with Click-iT™ EdU
PerCP, allophycocyanin (APC) and APC-based tandems (e.g., Alexa Fluor® 680-APC)	Completely compatible with Click-iT™ EdU
R-phycoerythrin (RPE) and RPE-based tandem conjugates (e.g., Alexa Fluor® 610-RPE)	Use RPE and RPE-based tandem conjugates after the Click-iT™ detection reaction

* Compatibility indicates whether the fluorescent molecule itself or the detection methods involve components that are unstable in the presence of the copper catalyst used for the Click-iT™ detection reaction. † Not all anti-GFP antibodies recognize the same antigen site. Rabbit and chicken anti-GFP antibodies perform well, whereas mouse monoclonal antibodies do not generate an acceptable amount of fluorescence and are not recommended for this application.

Product	Quantity	Cat. no.
Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay Kit, 10-plate size	1 kit	C10022
Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay Kit, 10-plate size	1 kit	C10033
Click-iT™ EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10027
Click-iT™ EdU Alexa Fluor® 488 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10028
Click-iT™ EdU Alexa Fluor® 594 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10209
Click-iT™ EdU Alexa Fluor® 647 High-Throughput Imaging (HCS) Assay Kit, 2-plate size	1 kit	A10208
Click-iT™ EdU Pacific Blue™ Flow Cytometry Assay, 50 assays	1 kit	A10034
Click-iT™ EdU Alexa Fluor® 488 Flow Cytometry Assay, 50 assays	1 kit	C35002
Click-iT™ EdU Alexa Fluor® 647 Flow Cytometry Assay, 50 assays	1 kit	A10202
Click-iT™ EdU Alexa Fluor® 488 Imaging Assay Kit, 50 coverslips	1 kit	C10083
Click-iT™ EdU Alexa Fluor® 594 Imaging Assay Kit, 50 coverslips	1 kit	C10084
Click-iT™ EdU Alexa Fluor® 647 Imaging Assay Kit, 50 coverslips	1 kit	C10085
EdU (5-ethynyl-2'-deoxyuridine)	50 mg	A10044

Phospholipidosis detection: Moving from coverslip to plate

Nioi, P., Perry, B.K., Wang, E.J., Gu, Y.Z., and Snyder, R.D., et al. (2007) *In vitro* detection of drug-induced phospholipidosis using gene expression and fluorescent phospholipid–based methodologies. *Toxicological Sci* 99:162–173.

Is there a rapid and robust method for high-throughput screening of potential phospholipidosis-inducing drugs? Phospholipidosis (PLD) is a disorder of lipid storage, characterized by the intracellular accumulation of excess phospholipids. A number of widely prescribed drugs, including phenobarbital and tamoxifen, are known to induce PLD; all are cationic amphiphilic drugs (CADs) containing a hydrophobic ring moiety and a charged amine. Although there is little evidence that drug-induced PLD represents a health hazard, it is a regulatory concern nonetheless, in part due to uncertainty about the precise effects of PLD at the cell and tissue level.

The currently accepted method for detecting PLD—electron microscopy—is unsuitable for high-throughput screening of drug candidates. In their recent study, Nioi and colleagues applied two distinct methods to the high-throughput detection of drug-induced PLD. Their first method, based on rtPCR analysis of PLD biomarkers, correctly identified 5 of 8 PLD-inducing drugs and 4 of 4 negative controls; the remaining three drugs were correctly identified by this method following an increase in the drug dose. This necessity for testing a range of drug concentrations, along with uncertainty about the specific mechanisms relating PLD-inducing drugs and gene expression, led the authors to look for a more direct assay method.

Their second approach, based on fluorescence detection using the LipidTOX™ fluorescently labeled phospholipid, correctly identified 100% of PLD-positive and -negative compounds at drug levels below those required for 100% identification via the gene expression analysis method. Although erythromycin and quinidine, both known PLD inducers, were not identified as positive using gene expression analysis, both were identified correctly by the LipidTOX™ assay even at concentrations as low as 25 µM (Figure 1). The observed fluorescence signal increased in a dose-dependent manner as the drug dosage was increased. Further, the authors were able to adapt the LipidTOX™ assay for use in plate-based fluorimetry suitable for large-scale drug screening, validating this PLD assay in a detection format that can provide greater high-throughput screening (HTS) capabilities than can automated imaging-based high-content screening (HCS) assays. The authors cite superior sensitivity, as well as speed and throughput capability, as strong factors in favor of using the LipidTOX™ stain–based method for the screening of potential PLD-inducing drugs. ■

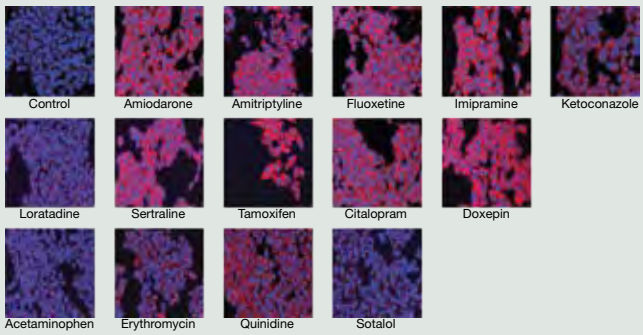


Figure 1—Treatment of HepG2 cells with PLD-inducing drugs causes the intracellular accumulation of a fluorescently labeled phospholipid. HepG2 cells were exposed for 48 hours to LipidTOX™ reagent and the indicated concentration of compound; amiodarone, 8.3 µM; amitriptyline, 25 µM; fluoxetine, 8.3 µM; imipramine, 25 µM; ketoconazole, 8.3 µM; loratadine, 8.3 µM; sertraline, 8.3 µM; tamoxifen, 8.3 µM; citalopram, 25 µM; doxepin, 25 µM; acetaminophen, 25 µM; erythromycin, 25 µM; quinidine, 25 µM; sotalol, 25 µM. Confocal images of fixed cells stained with Hoechst are shown. Nuclei are stained blue and fluorescently labeled phospholipid is red. From Nioi, P. et al., *In vitro* detection of drug-induced phospholipidosis using gene expression and fluorescent phospholipid–based methodologies, *Toxicological Sci* (2007) 99:162–173, used by permission of Oxford University Press.

Product	Quantity	Cat. no.
HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit, for high-content screening and cellular imaging, 2-plate size	1 kit	H34157
HCS LipidTOX™ Phospholipidosis and Steatosis Detection Kit, for high-content screening and cellular imaging, 10-plate size	1 kit	H34158
HCS LipidTOX™ Green phospholipidosis detection reagent, 1000X aqueous solution, for cellular imaging, 10-plate size	each	H34350
HCS LipidTOX™ Red phospholipidosis detection reagent, 1000X aqueous solution, for cellular imaging, 10-plate size	each	H34351
HCS LipidTOX™ Green neutral lipid stain, solution in DMSO, for cellular imaging	each	H34475
HCS LipidTOX™ Red neutral lipid stain, solution in DMSO, for cellular imaging	each	H34476
HCS LipidTOX™ Deep Red neutral lipid stain, solution in DMSO, for cellular imaging	each	H34477

Potassium flux assay meets BacMam technology

COMBINING SENSITIVE FLUXOR™ FLUORESCENCE DETECTION WITH EFFICIENT BACMAM-MEDIATED DELIVERY.

Ion channels are a diverse class of proteins that can be divided into two main categories—ligand-gated ion channels and voltage-gated ion channels—where the transition between the open and closed states is regulated by ligands and by cellular membrane potential, respectively.[1,2] Although ion channels are separated into distinct groups based on structure and function, all are transmembrane protein complexes that form an aqueous pore through which ions can enter and exit cells according to the prevailing electrochemical gradient. This characteristic makes ion channels important targets in a number of therapeutic areas and disease states,[3,4] including immune response mechanisms, pain, diabetes, and hypertension. At the same time, the central role of ion channels in cell physiology [4,5] imparts critical importance to the assessment of off-target drug interactions with ion channels in drug safety testing.[6]

Significant efforts have been dedicated to developing tools for high-throughput screening (HTS) of compounds involved in ion channel drug discovery. Current ion channel assay techniques such as manual and automatic patch clamping, ligand-binding assays, dye-based membrane potential measurements using voltage-sensitive probes, and flux assays using surrogate ions such as rubidium (Rb I) [7–9] are relatively time-consuming and costly. The FluxOR™ Thallium Detection Kit offers researchers a new method for screening voltage-gated potassium channels based on measuring the flux of a surrogate ion. By combining the sensitive FluxOR™ thallium detection assay with efficient BacMam-mediated delivery and expression of ion channels such as the hERG potassium channel, researchers can now achieve reproducible, high-throughput measurements of potassium flux.

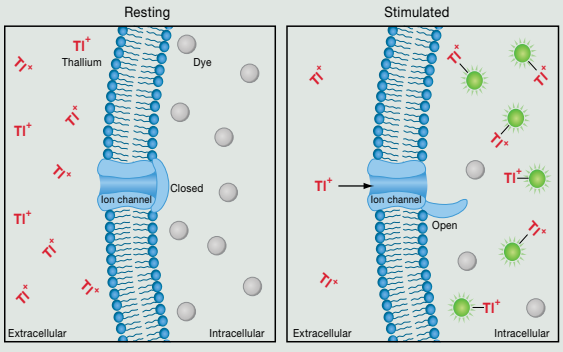


Figure 1—Principle of the FluxOR™ assay. Basal fluorescence from cells loaded with the FluxOR™ dye is low **(A)** until potassium channels are stimulated. Thallium (I) ions added to the assay with the stimulus flow into the cells, activating the dye **(B)**. The high sensitivity of the FluxOR™ dye gives an excellent signal window for detecting potassium channel activity.

The FluxOR™ assay: A new method for measuring potassium flux

The FluxOR™ thallium detection assay is based on the novel method developed by Weaver et al. [10] for screening voltage-gated potassium channels using a fluorescent sensor dye with an affinity for thallium (I) ions, which function as potassium ion surrogates; the assay measures the flux of the surrogate ion. This homogenous potassium channel activity assay takes advantage of the permeability of potassium channels and transporters to thallium ions;[10,11] when channels are opened by a stimulus, thallium influx from the external medium is detected with a highly sensitive indicator dye (Figure 1). The resulting fluorescence quantitatively reflects the activity of ion channels and transporters that are permeant to thallium, including hERG, Kir2.1, KATP, and other pharmacologically important targets. Using this assay, inhibition curves approach the sensitivity of conventional patch clamp methods. The fluorescence-based thallium flux assay was recently used in conjunction with patch-clamp experiments in an elegant study of selective modulation of SK2 and SK3 subtypes of Ca^{2+}-activated potassium channels,[12] demonstrating the compatibility of the two methods.

The FluxOR™ Thallium Detection Kit allows high-throughput screening of potassium channel targets with real-time results, reproducibly giving IC_{50} values comparable to those obtained using

lower-throughput methods. The FluxOR™ assay should be useful with a variety of voltage and ligand-gated potassium channels, and should prove adaptable to any process that passes thallium into the cytosol in an activity-dependent manner as a result of flux or transport.

FluxOR™ assay principle

The FluxOR™ reagent, a thallium indicator dye, is loaded into cells as a membrane-permeable AM ester. Loading is assisted by the PowerLoad™ concentrate included in the kit, a formulation of Pluronic® surfactants (BASF Corporation) that disperse and stabilize AM ester dyes for optimal loading in aqueous solution. Intracellular FluxOR™ dye is then cleaved by endogenous esterases into a highly thallium-sensitive reporter. The thallium-responsive form is trapped in the cytosol, its extrusion prevented by water-soluble probenecid that blocks organic anion pumps. When a small amount of thallium is added to the cells along with a stimulus solution that opens potassium-permeant ion channels, thallium enters the cells. Upon binding cytosolic thallium, the de-esterified FluxOR™ dye exhibits an increase in fluorescence (emission ~525 nm). Monitoring fluorescence gives a real-time, functional readout of thallium redistribution.

A highly sensitive, chloride-compatible potassium flux assay

The sensitivity of the FluxOR™ dye permits the use of low levels of extracellular thallium; millimolar concentrations give large signals in HTS mode, allowing the dye to be used in physiological saline with no need to load or assay cells in chloride-free buffer. This is a great advantage compared to traditional thallium flux assays, which require completely chloride-free conditions to load cells with the dye.[13] Thallium chloride is an insoluble precipitate that forms when concentrations of free thallium and chloride in the solution are greater than ~4 mM. Because the FluxOR™ reagent is extremely sensitive to thallium, extracellular thallium can be used at concentrations less than 4 mM in the stimulus buffer, or ~2.8 mM after a 1.5 dilution in a typical HTS assay. For ligand-gated channels, resting potassium channels, and potassium transporters, the potassium normally added to the stimulus buffer to activate voltage-gated channels is not needed. For low-expressing or poorly thallium-permeant targets requiring high extracellular thallium concentrations, enough chloride-free buffer is supplied for the assay and stimulation, allowing maximum ease and flexibility of use against a wide range of targets.

FluxOR™ detection meets BacMam expression and delivery

Like other pharmacological targets, ion channel targets have traditionally been screened in cells constructed to stably express the protein at a high, constitutive level. However, this approach has proven to be challenging with this class of proteins.[13–15] Researchers are increasingly turning to inducible, division-arrested, or transient expression systems to overcome toxicity, clonal drift, and other cellular inconsistencies that hamper successful experimentation and screening of relevant targets. BacMam technology, a straightforward method of gene delivery and expression in mammalian cells by a baculovirus,[16] offers few to no observable side effects and has been used successfully in pharmacologically relevant screens of ion channels and transporters.[6,7,11] The flexibility of BacMam delivery technology in delivering multiple constructs at different stoichiometries and subunit compositions is a benefit for some members of these complex protein families.[13,15,16]

The BacMam delivery system uses a modified insect cell baculovirus as a vehicle to efficiently deliver and express genes in mammalian cells. Because baculoviruses are insect cell viruses, only the transgenes associated with mammalian regulatory elements are expressed; expression of the hERG gene is driven by the CMV promoter (Figure 2). →

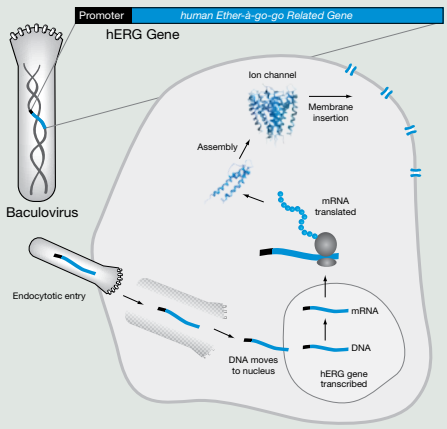


Figure 2—BacMam-hERG gene delivery and expression. This schematic depicts the mechanism of BacMam-mediated gene delivery into a mammalian cell and expression of the hERG gene. The hERG gene resides within the baculoviral DNA, downstream of a CMV promoter which drives its expression when introduced into a mammalian target cell. BacMam viral particles are taken up by endocytic pathways into the cell, and the DNA within them is released for transcription and expression. The translated protein is then folded for insertion into the membrane, forming functional hERG ion channels. This process begins within 4–6 hours and in many cell types is completed after an overnight period.

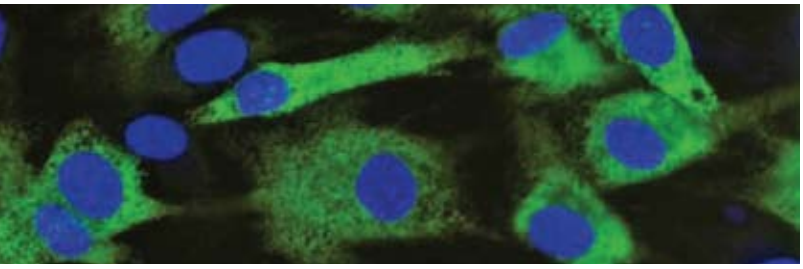


Figure 3—Human arterial smooth muscle cells (HASMC; Cat. no. C-007-5C) following transduction with Organelle Lights™ Mito-GFP (Cat. no. O36210). The image shows cells after incubation for 12 days. Cells were stained with DAPI and images were visualized using a fluorescence microscope at 40x magnification.

The virus does not replicate and no viral genes are expressed, making baculoviruses both safe and noncytopathic.[17] The efficient BacMam transduction is readily performed using automated liquid handling, which affords excellent consistency within and between plates. The large number of cell types that can be efficiently transduced, the ability to deliver multiple BacMam reagents, and the ease of gene expression modulation are features that in combination give BacMam technology unique benefits in a discovery setting.

BacMam-mediated delivery provides efficient and reproducible transduction in a broad range of cell types (excluding cells of hematopoietic origin), including primary and stem cells, without apparent cytopathic effects. Gene expression levels are easily modulated by varying the titer of the BacMam reagent to optimize assay conditions. In addition, factors such as enhancer volume, cell confluence, and incubation time can be used to vary gene expression. Batch transduction provides a homogenous cell population approximating the consistency of stable cell lines without the risk of genetic drift. Expression of the fluorescent protein is unaffected by freezing, and upon plating cells, can remain brightly stained for more than 120 hours depending on cell line; we have demonstrated expression of Organelle Lights™ reagents for up to 12 days in Cascade Biologics® primary cells (Figure 3). For a further discussion of BacMam technology advantages, see pages 26–28.

The BacMam-hERG system

To enable the study of ion channels in a variety of mammalian cells, we engineered potassium channel cDNA into an insect cell virus (baculovirus) chromosome behind a mammalian control element (the CMV promoter) using BacMam technology (Figure 2). BacMam potassium channel constructs that have been developed and functionally verified with the FluxOR™ system include Kir2.1, KATP, and the human ether-a-go-go related gene (hERG, or human Kv11.1). The BacMam–hERG system provides a method to reproducibly measure potassium flux in a variety of null-background cellular models, including human U-2 OS cells (ATCC HTB-96, Figure 4). The BacMam–hERG system gives transient expression of hERG in U-2 OS cells with high (>90%) efficiency, and is fully compatible with the FluxOR™ Thallium Detection Kit.

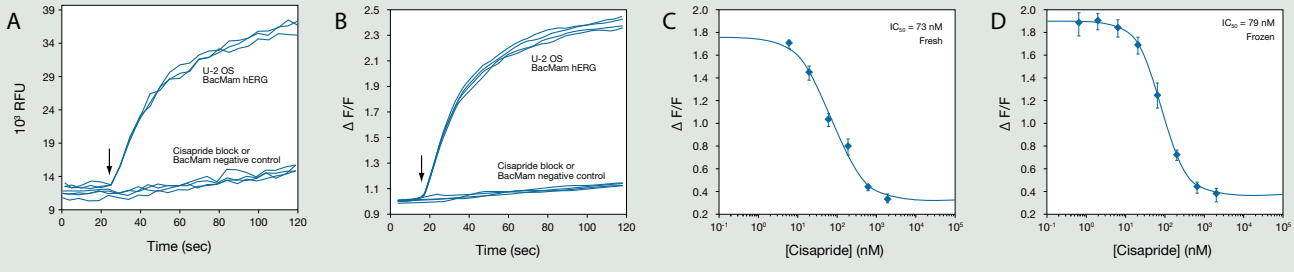


Figure 4—FluxOR™ thallium flux assays performed on fresh and frozen U-2 OS cells transduced with BacMam-hERG. (A) Raw data obtained in the FluxOR™ assay determination of thallium flux in U-2 OS cells transduced with BacMam-hERG and kept frozen until the day of use. The arrow indicates the addition of the thallium/potassium stimulus, and upper and lower traces indicate data taken from the minimum and maximum doses of cisapride used in the determination of the dose-response curves. **(B)** Raw pre-stimulus peak and baseline values were boxcar averaged and normalized, and indicate the fold increase in fluorescence over time. **(C)** Data generated in a dose-response determination of cisapride block on BacMam hERG expressed in U-2 OS cells freshly prepared from overnight expression after viral transduction. **(D)** Parallel data obtained from cells transduced with BacMam-hERG, stored for 2 weeks in liquid nitrogen, thawed, and plated 4 hours prior to running the assay. Error bars indicate standard deviation, n = 4 per determination.

Cell lines compatible with BacMam technology

Other cell lines that are efficiently transduced by BacMam technology include HEK293, HepG2, BHK, Cos-7 and Saos-2. The FluxOR™ Thallium Detection Kit has been demonstrated with hERG-T-REx™ 293 (Cat. no. K1236) and CHO (Cat. no. K1237) cell lines, which express a tetracycline-inducible clone of hERG. The FluxOR™ assay in combination with BacMam-mediated potassium channel delivery has also been used in Cascade Biologics® human primary cells, including human arterial smooth muscle cells (HASMC; Cat. no. C-007-5C) and human pulmonary arterial vein endothelial cells (HPAEC; Cat. no. C-008-5C). And although the FluxOR™ assay was optimized using BacMam-hERG as a model system, this method is useful for other potassium channels, including Kir2.1 (Figure 5).

Preparing drug libraries for the FluxOR™ assay

Compound libraries or dose-response series can either be prepared in a stimulus buffer for concomitant addition with the stimulus or be added in a pretreatment step; effective compound concentrations at target ion channels may be different for each condition. Because the FluxOR™ assay is an equilibrium measurement in which thallium redistributes across the cell membrane through channels with complex gating mechanisms, effective concentrations of drug block obtained with this procedure may not match those obtained by patch clamp measurements. However, rank-order potency will generally reflect that obtained by electrophysiological measurements. The FluxOR™ Thallium Detection Kit is a screening, pharmacology, and hit-identification tool and is not intended to replace patch clamp safety testing of drug activity at hERG potassium channels.

Sensitive, efficient high-throughput screening of potassium flux

The FluxOR™ Thallium Detection Kit and the BacMam–hERG system are available individually, or combined in the FluxOR™ Thallium Expression and Detection system. Together, these products enable true high-throughput functional screening of potential hERG agonists under physiological conditions, without the need for quenchers or radioisotopes. To learn more about the FluxOR™ Thallium Detection Kit and BacMam-mediated delivery, visit **www.invitrogen.com/fluxor**. ■

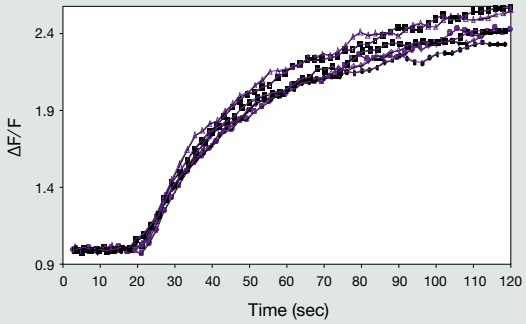


Figure 5—Potassium flux in frozen/thawed primary human arterial smooth muscle cells transduced with BacMam-Kir2.1. Primary human arterial smooth muscle cells were batch transduced with BacMam-Kir2.1. Following incubation overnight, cells were split into aliquots and stored frozen at –80°C until use. On the day of the assay, cells were thawed and plated, and the FluxOR™ thallium detection assay was run 5 hours later.

References

1. Doyle, D.A. (2004) *Eur J Biophys* 33:175–179.
2. Jenkinson, D.H. (2006) *Br J Pharmacol* 147 Suppl 1:S63–S71.
3. Ashcroft, F.M. (1999) *Ion Channels and Disease: Channelopathies*, San Diego, CA:Academic Press.
4. Clare, J.J. and Trezise, D.J., eds. (2006) *Expression and Analysis of Recombinant Ion Channels: From Structural Studies to Pharmacological Screening*, Weinheim, Germany: Wiley-VCH.
5. Lawson, K. and McKay, N.G. (2006) *Curr Pharm Des* 12:459–470.
6. Thomas, D. et al. (2006) *Curr Pharm Des* 12:2271–2283.
7. Gill, S. et al. (2003) *Assay Drug Dev Technol* 1:709–717.
8. Inglese, J. et al. (2007) *Nature Chem Biol* 3:466–479.
9. Zheng, W. et al. (2004) *Assay Drug Dev Technol* 2:543–552.
10. Weaver, C.D. et al. (2004) *J Biomol Screen* 9:671–677.
11. Hille, B. (1973) *J Gen Physiol* 61:669–686.
12. Hougaard, C. et al. (2007) *British J Pharmacol* 151:655–665.
13. Condreay, J.P. et al. (2006) *Adv Virus Res* 68:255–286.
14. Pfohl, J.L. et al. (2002) *Receptors Channels* 8:99–111.
15. Clare, J.J. (2006) Functional Expression of Ion Channels in Mammalian Systems. In *Expression and Analysis of Recombinant Ion Channels: From Structural Studies to Pharmacological Screening*, Clare, J.J. and Trezise, D.J., eds. Weinheim, Germany: Wiley-VCH, pp. 79–109.
16. Ames, R.S. et al. (2007) *Expert Opin Drug Discov* 2:1669–1681.
17. Kost, T.A. et al. (2006) Biosafety and viral gene transfer vectors. In *Biological Safety: Principles and Practices*, 4th Ed., Fleming, D.O. and Hunt, D.L., eds., Washington, D.C.:ASM Press, pp. 509–529.

Product	Quantity	Cat. no.
BacMam-hERG, for 10 microplates	1 kit	B10019
BacMam-hERG, for 100 microplates	1 kit	B10033
FluxOR™ Thallium Detection Kit, for 10 microplates	1 kit	F10016
FluxOR™ Thallium Detection Kit, for 100 microplates	1 kit	F10017
FluxOR™ Thallium Expression and Detection Kit with BacMam-hERG, for 10 microplates	1 kit	F10030
FluxOR™ Thallium Expression and Detection Kit with BacMam-hERG, for 100 microplates	1 kit	F10032
PowerLoad™ concentrate, 100x	1 kit	P10020

Qdot® 625 nanocrystals and conjugates

A BETTER, BRIGHTER RED NANOCRYSTAL FOR IMMUNOSTAINING, FLOW CYTOMETRY, AND MORE.

The first of our next generation quantum dots, the Qdot® 625 nanocrystals, are now available from Invitrogen. This new color complements our series of visible and near-infrared colors of Qdot® nanocrystals (Figure 1), making the possibility of true eight-color multispectral analyses a reality for many researchers. Qdot® 625 nanocrystals exhibit the same exceptional photostability and narrow emission profile as the other Qdot® nanocrystal colors but without the "red shoulder" often observed with traditional dyes (Figure 2). As evidenced by the extinction coefficients using 405 nm and 488 nm excitation, Qdot® 625 nanocrystals are the brightest of the visible range quantum dots (Table 1).

Qdot® 625 nanocrystals for a multitude of applications

Our Qdot® 625 streptavidin and goat anti–mouse IgG and anti–rabbit IgG secondary antibody conjugates provide a high level of resolution and brightness for immunostaining of cells grown in culture (Figure 3). For optimal detection of the Qdot® 625 nanocystals, we recommend the BrightLine® QD625-A Filter Set (www.semrock.com/Catalog/Brightline_QD625.htm). The Qtracker® 625 Cell Labeling Kit provides researchers with the means to nonspecifically load Qdot® nanocrystals into living cells from culture for long-term cell tracking. Invitrogen has tested this new material in both CHO and HeLa cells, and from experience with our other Qdot® colors used for cell labeling, many other cell types should also be compatible with the 625 dots. In addition, Qdot® 625 conjugates are compatible with flow cytometry, and exhibit their best utility when used in instruments equipped with violet lasers (Figure 4).

Because the electron-dense Qdot® nanocrystals have uniform size distribution and shape, some researchers have successfully correlated fluorescence data with electron microscopy data to resolve the spatial location of targets immunostained with Qdot® conjugates.[1] This utility is further exploitable because some of the Qdot® nanocrystals themselves have distinguishable shapes (Qdot® 605 and Qdot® 655 are rod shaped, whereas the rest of the Qdot® nanocrystals, including Qdot® 625, are spherical (Figure 5)), making resolution of dual-stained samples at the near-molecular level a possibility.

The compatibility of Qdot® 625 nanocrystals with existing Qdot® colors makes it possible to perform multispectral analysis with all eight

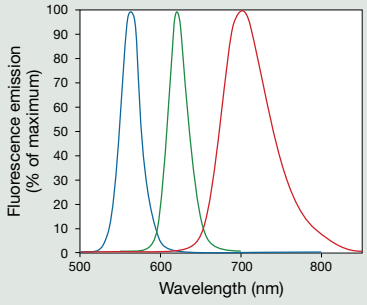


Figure 1—Fluorescence emission spectra of Qdot® 565, Qdot® 625 and Qdot® 705 streptavidin conjugates. In the context of multiplex detection with Qdot® 565 and Qdot® 705 conjugates, the relative position of the Qdot® 625 emission envelope is superior in terms of signal crosstalk to Qdot® 605 or Qdot® 655 conjugates. In such applications, all Qdot® conjugates can be efficiently excited at a single wavelength in the near-UV/violet region (e.g., 405 nm).

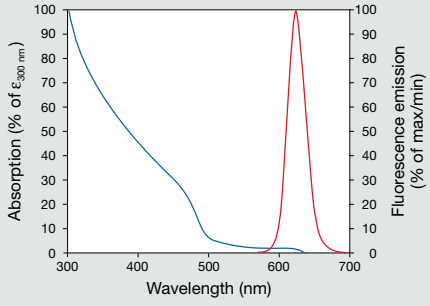


Figure 2—Absorption and emission spectra of Qdot® 625 streptavidin conjugate.

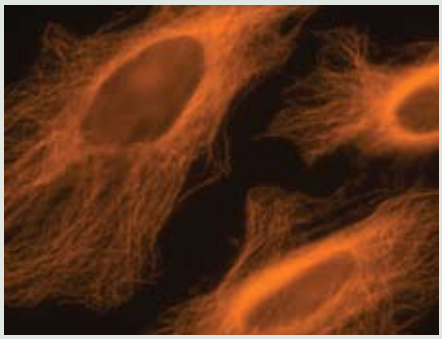


Figure 3—Qdot® 625 goat anti-mouse conjugate immunolabeling of microtubules in HeLa cells. Fixed and permeabilized HeLa cells were labeled with mouse anti–α-tubulin primary antibody and 20 nM Qdot® 625 goat anti–mouse IgG.

Table 1—Extinction coefficient of Qdot® streptavidin conjugates at common excitation wavelengths, in $cm^{-1}M^{-1}$. For comparison, the extinction coefficient of fluorescein with 488 nm excitation is ~80,000 $cm^{-1}M^{-1}$.

Product	405 nm	488 nm
Qdot® 525 nanocrystals	360,000	130,000
Qdot® 565 nanocrystals	1,100,000	290,000
Qdot® 585 nanocrystals	2,200,000	530,000
Qdot® 605 nanocrystals	2,800,000	1,100,000
Qdot® 625 nanocrystals	9,900,000	2,700,000
Qdot® 655 nanocrystals	5,700,000	2,900,000

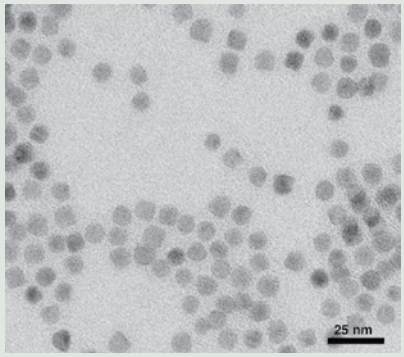


Figure 5—Qdot® 625 streptavidin transmission electron microscopy (TEM) images. Courtesy of Mark Ellisman, National Center for Microscopy and Imaging Research, University of California San Diego, San Diego, CA.

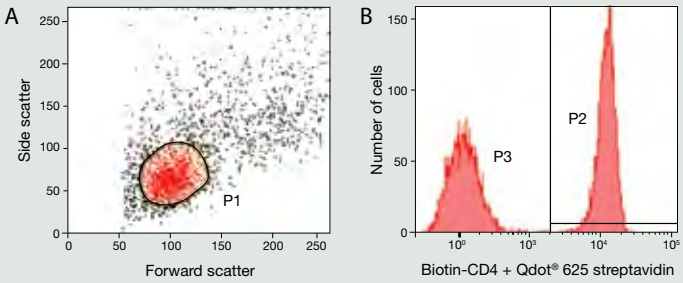


Figure 4—Qdot® 625 streptavidin conjugate for the characterization of human lymphocytes using flow cytometry. Human lymphocytes were first labeled with a mouse anti–human CD4 biotin conjugate. After washing with 1% BSA/PBS, the cells were then incubated with Qdot® 625 streptavidin conjugate for 15 minutes at room temperature. Cells were washed again with 1% BSA/PBS and analyzed in LSR II flow cytometer (BD Biosciences). When gated on lymphocytes (P1 in panel **A**), the histogram **(B)** shows very good peak separation between the CD4-positive (P2) and negative (P3) populations. The PMT setting was 500 V, and a 600 nm longpass dichroic mirror and a 630/22 nm bandpass filter were used. For red-excited fluorophores, a shorter wavelength bandpass filter can be used.

Qdot® nanocrystal colors using a single excitation source. The exceptional brightness exhibited by Qdot® 625 nanocrystals offers a distinct advantage when detecting low-abundance targets. These materials may also prove useful for new areas of bioresearch that involve investigating the special location and tracking of single molecules in living cells. Learn more about the advantages of Qdot® nanocrystals at **www.invitrogen.com/qdots**. ■

Reference

1. Giepmans, B.N. et al. (2005) *Nat Methods* 2:743–749.

Product	Quantity	Cat. no.
Qdot® 625 streptavidin conjugate, 1 µM solution	200 µl	A10196
Qdot® 625 goat F(ab')$_2$ anti–mouse IgG conjugate (H+L), 1 µM solution, highly cross-adsorbed	100 µl	A10195
Qdot® 625 goat F(ab')$_2$ anti–rabbit IgG conjugate (H+L), 1 µM solution, highly cross-adsorbed	100 µl	A10194
Qtracker® 625 Cell Labeling Kit	1 kit	A10198
Qdot® 625 ITK™ carboxyl quantum dots, 8 µM solution	250 µl	A10200

Nuclear receptor antibodies: A core technology

CHOOSE FROM ANTIBODIES TO A WIDE RANGE OF NUCLEAR RECEPTOR TARGETS.

Nuclear receptors (NR) are a superfamily of ligand-activated transcription factors that mediate cellular responses to a broad range of small molecular weight nonpeptide signals, including endogenous hormones and metabolites and xenobiotic compounds. There are at least 48 NRs in humans, and physiologically relevant ligands have been identified for about half of these receptors; the remainder are referred to as orphan receptors. Invitrogen now offers a portfolio of human NR antibodies from Perseus Proteomics, Inc.

Human NR antibodies from Perseus Proteomics

These 66 human NR antibodies were developed using the baculovirus expression technique by Takao Hamakubo at the University of Tokyo. This technology, which is licensed to Perseus Proteomics, displays the target protein in its native structure on the surface of budded baculoviruses, which makes the protein more directly accessible as an antigen (Figure 1). This accessibility enables the development of antibodies against proteins with more complex structures, which were previously difficult to produce. The NR antibodies have been validated for use in multiple applications, including western blotting, ELISAs, immunofluorescence, immunohistochemistry (Figure 2), electrophoretic mobility-shift assays (EMSAs), and immunocytochemistry. All of the antibodies react with human proteins, and many react with other species as well.

Complete NR antibody portfolio

Invitrogen offers antibodies to a broad range of NR targets, including estrogen receptors, progesterone receptors, and glucocorticoid receptors. View our complete listing of nuclear receptor antibodies at **www.invitrogen.com/nuclearreceptorantibodies**. ■

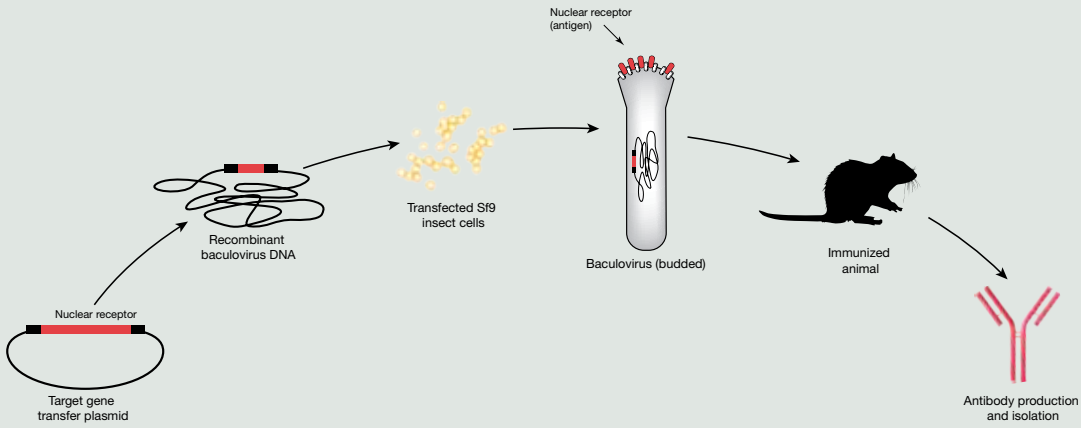


Figure 1—The baculovirus expression system. Using the baculovirus expression technique, large amounts of target protein can be displayed in their native structure on the surface of budded baculoviruses, and are directly available as antigens. The baculovirus expression system makes it possible to raise high-affinity antibodies against membrane proteins with complex structures.

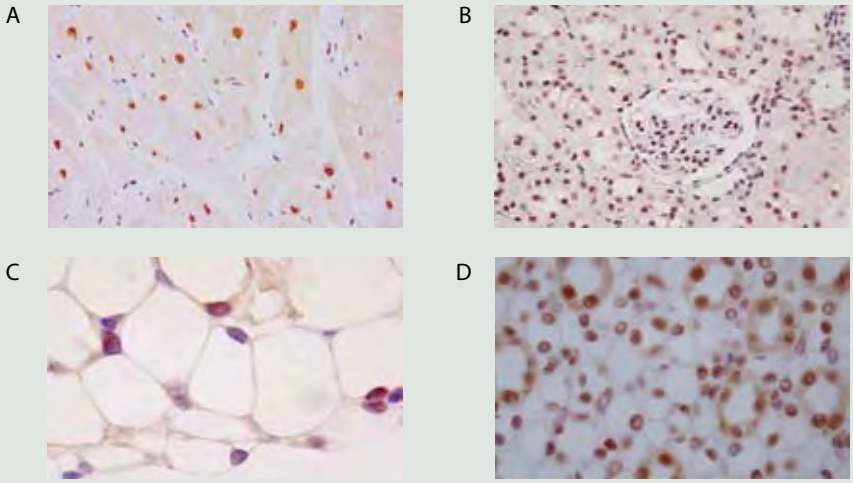


Figure 2—Immunohistochemical analysis of samples stained with nuclear receptor antibodies. (A) Human heart myocardium stained with mouse anti-human ERRα (Cat. no. 416600). **(B)** Rat kidney stained with mouse anti-human GR common (clone H8004) (Cat. no. 417500). **(C)** Rat adipose tissue stained with mouse anti-human PPARγ common (clone A3409A) (Cat. no. 419300). **(D)** Rat kidney collecting tubules stained with mouse anti-human ROR common (Cat. no. 433500).

Product *	Isotype	Clone/PAD	Reactivity	Applications	Cat. no.
Mouse anti-human CAR	Mouse IgG1	N4111	Hu	WB, E, IP	416000
Mouse anti-human COUP-TF I	Mouse IgG2a	H8132	Hu, Ms, Rt	WB, E, IP, IHC	416100
Mouse anti-human COUP-TF II	Mouse IgG2a	H7147	Hu, Ms, Rt	WB, E, IP, IHC	416200
Mouse anti-human DAX1	Mouse IgG2a	H7431	Hu	WB, E	416300
Mouse anti-human ERRα	Mouse IgG2a	H5844	Hu, Rt	WB, E, IP, IHC	416600
Mouse anti-human FXR	Mouse IgG2a	A9033A	Hu, Ms, Rt	WB, E, IP, IHC	417200
Mouse anti-human GR common (clone H8004)	Mouse IgG2a	H8004	Hu, Rt	WB, E, IP, IHC	417500
Mouse anti-human HNF4α (clone K9218)	Mouse IgG2a	K9218	Hu, Ms, Rt	WB, E, IP, IHC, EMSA	417600
Mouse anti-human HNF4α (clone H1415)	Mouse IgG2a	H1415	Hu, Ms, Rt	WB, E, IP, EMSA, ChIP, IHC, IF	417700
Mouse anti-human LRH-1 (clone H2325)	Mouse IgG3	H2325	Hu, Ms, Rt	WB, E, IP	418100
Mouse anti-human LXRα	Mouse IgG2a	K8607	Hu	WB, E, EMSA, IP, ChIP	418200
Mouse anti-human LXRα ligand binding domain	Mouse IgG2a	PPZ0412	Hu, Rt	WB, E, IP, ChIP, EMSA, IHC	418300
Mouse anti-human NGFI-Bα	Mouse IgG2a	H1648	Hu	WB, E, IP	418600
Mouse anti-human NGFI-Bβ	Mouse IgG2a	N1404	Hu, Rt	WB, E, IP, IHC	418700
Mouse anti-human PPARα	Mouse IgG2a	H0723	Hu, Ms	WB, E, IP, ChIP, EMSA	419000
Mouse anti-human PPARγ common (clone K8713)	Mouse IgG2a	K8713	Hu, Ms	WB, E, IP, EMSA, ChIP	419200
Mouse anti-human PPARγ common (clone A3409A)	Mouse IgG2a	A3409A	Hu, Ms, Rt	WB, E, IP, EMSA, ChIP, IHC	419300
Mouse anti-human PPARδ	Mouse IgG2a	K9436	Hu	WB, E, EMSA	419400
Mouse anti-human PR common	Mouse IgG2a	A9621A	Hu, Rt	WB, E, IP, IHC	419500
Mouse anti-human REVERBα	Mouse IgG2a	A8740A	Hu	WB, E, IP	433300
Mouse anti-human ROR common	Mouse IgG2a	H3925	Hu, Rt	WB, E, IP, IHC	433500
Mouse anti-human RORα	Mouse IgG2a	H3910	Hu	WB, E, IP	433600
Mouse anti-human SF-1	Mouse IgG1	N1665	Hu, Rt	WB, E, IP, IHC	434200

* Each antibody is provided in a unit size of 100 µg. Hu = human; Ms = mouse; Rt = rat; WB = western blot; E = ELISA; IP = immunoprecipitation; IHC = immunohistochemistry; EMSA = electrophoretic mobility-shift assays; ChIP = chromatin immunoprecipitation; IF = immunofluorescence.

Flow cytometry: checking vital signs

A SURVEY OF MOLECULAR PROBES® VIABILITY AND VITALITY TECHNOLOGIES.

Viability and cytotoxicity assays are principally used to enumerate the proportion of live and dead cells in a population, and most commonly rely on a simple check of membrane integrity. However, cell vitality comprises a range of cell interactions with the environment, and can be judged based on virtually any cell process from membrane function to proliferative capacity (Figure 1). The diversity of live cells and their environments makes it impossible to devise a single assay applicable to all cell types, and assays deemed useful must also meet the needs of individual researchers. Invitrogen offers a range of flow cytometry assays that can be used with available lasers and emission channels.

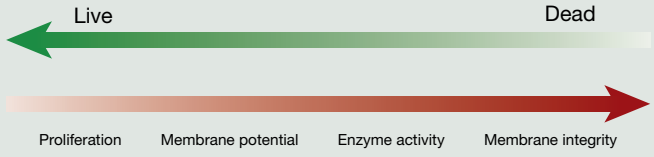


Figure 1—Cell viability comprises a spectrum of cell responses to the environment.

Membrane-impermeant DNA dyes

Loss of membrane integrity is the ultimate indicator of cell death, and is easily detected with impermeant DNA dyes. These dyes are excluded from cells with intact membranes, but enter cells with compromised

Table 1—Spectral characteristics of reagents used for viability and vitality measurements.

Excitation source	UV	405 nm			488 nm	
Emission color	Blue	Blue	Green	Yellow	Green	Yellow
Impermeant dyes	• DAPI (D3571)	• SYTOX® Blue dye (S34857)	• SYTOX® Blue dye (S34857)		• SYTOX® Green dye (S7020)	• Dead cell discriminator (DCD00) • Propidium iodide (P3566)
Fixable dead-cell dyes	• Fixable blue dye (L23105)	• Fixable violet dye (L34955)	• Fixable aqua dye (L34957)		• Fixable green dye (L23101)	
Mitochondrial membrane potential					• JC-1 (M34152) • $DiOC_2(3)$ (M34150) • Rhodamine 123 (R302)	• JC-1 (M34152) • $DiOC_2(3)$ (M34150) • MitoTracker® Red CMXRos (M7512)
Metabolic and enzymatic activity	• CellTrace™ calcein blue AM (C34853)	• CellTrace™ calcein violet AM (C34858)			• Calcein AM (C3100MP)	• C_{12}-resazurin (V23110) • CellTrace™ calcein red-orange AM (C34851)
Other cell responses (calcium flux, pH)	• Indo-1 AM (I1223)			• Fura Red™ AM (F3021)	• Fluo-3 AM (F1242) • Fluo-4 AM (F14201)	• Fura Red™ AM (F3021) • SNARF®-1 AM dye (C1272)
Cell division: nucleoside incorporation		• Click-iT™ EdU Pacific Blue™ (A10034)			• Click-iT™ EdU Alexa Fluor® 488 (C35002) • Alexa Fluor® 488 anti-BrdU (A21303) • FITC anti-BrdU (MD5401) • ABSOLUTE-S™ SBIP (A23150)	
Cell division: cell cycle for intact cells	• Hoechst 33342 (H3569)	• DyeCycle™ Violet dye (V35003)			• DyeCycle™ Green dye (V35004)	• DyeCycle™ Orange dye (V35005)
Cell division: cell cycle for permeabilized cells	• DAPI (D3571) • Hoechst 33342 (H3569)	• DyeCycle™ Violet dye (V35003) • SYTOX® Blue dye (S34857)	• SYTOX® Blue dye (S34857)		• DyeCycle™ Green dye (V35004) • SYTOX® Green dye (S7020)	• DyeCycle™ Orange dye (V35005) • Propidium iodide (P3566)
Cell division: tracer dye analysis					• CFSE (C34554) • Oregon Green® SE (C34555) • DiO (V22886)	
Cell count	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• Caltag™ Counting Beads (PCB-100) • CountBright™ beads (C36950)	• Caltag™ Counting Beads (PCB-100) • CountBright™ beads (C36950)

Red = compatible with fixation. Example product numbers are provided; additional configurations may be found at www.invitrogen.com.

membranes and undergo significant fluorescence enhancement when they bind DNA. Propidium iodide (PI) is the most common dye in this group, but there are other dyes for the verification of membrane integrity that fit with every excitation source (Table 1). These dyes are often used in a "dump channel," with gating on the viable cells for further analysis (Figure 2). Because these dyes bind in equilibrium with DNA, external dye concentration must be maintained during analysis, and the dye should not be washed out. Impermeant DNA dyes are not compatible with fixation or intracellular staining protocols when used as viability probes.

Fixable dead-cell dyes

The fixable dead-cell dyes, which covalently interact with available amino groups, are a new class of viability dyes. Like impermeant dyes, the fixable dyes are excluded from the cytosol of healthy cells. The dyes react with surface proteins of healthy cells, but also label proteins throughout the cytoplasm of cells with damaged membranes, causing dead cells to have at least 50-fold greater fluorescence than live cells. Because the labeling is covalent, labeled cells can be aldehyde fixed and permeabilized without losing viability →

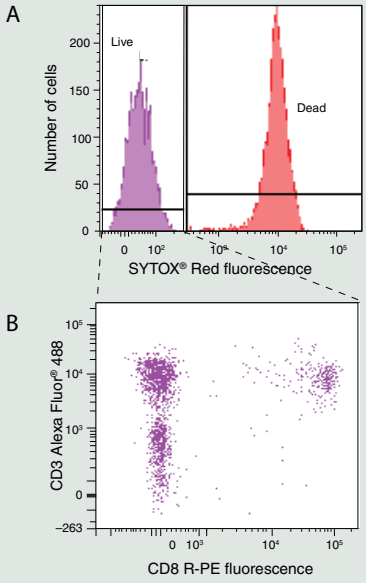


Figure 2—Viable cell gating with an impermeant DNA dye. A mixture of heat-treated and untreated human peripheral blood leucocytes (PBL) was stained with antibody conjugates, then stained with 5 nM SYTOX® Red stain before being analyzed by flow cytometry with 488 nm and 635 nm excitation. The dot plot showing cells stained for CD3 and CD8 antigens **(B)** was gated on live cells (left peak from the SYTOX® Red stain, panel **A**).

		532 nm			633 nm	
Orange	Red	Yellow	Orange	Red	Red	Infrared
• 7-AAD (A1310) • Dead cell discriminator (DCD00) • Propidium iodide (P3566)	• 7-AAD (A1310) • Dead cell discriminator (DCD00) • Propidium iodide (P3566)	• Dead cell discriminator (DCD00) • Propidium iodide (P3566)	• 7-AAD (A1310) • Dead cell discriminator (DCD00) • Propidium iodide (P3566) • SYTOX® Orange dye (S11368)	• 7-AAD (A1310) • Dead cell discriminator (DCD00) • Propidium iodide (P3566)	• SYTOX® Red dye (S34859)	
• Fixable red dye (L23102)			• Fixable red dye (L23102)		• Fixable far red dye (L10120)	• Fixable near IR dye (L10119)
• JC-1 (M34152) • $DiOC_2(3)$ (M34150)		• MitoTracker® Red CMXRos (M7512)			• $DiIC_1(5)$ (M34151)	
		• C_{12}-resazurin (V23110)				
• SNARF®-1 AM dye (C1272)		• Fura Red™ AM (F3021) • SNARF®-1 AM dye (C1272)	• SNARF®-1 AM dye (C1272)			
					• Click-iT™ EdU Alexa Fluor® 647 (A10202) • Alexa Fluor® 647 anti-BrdU (A21305)	• Alexa Fluor® 680 anti-BrdU (A31859)
		• DyeCycle™ Orange dye (V35005)				
• 7-AAD (A1310) • Propidium iodide (P3566)	• 7-AAD (A1310) • Propidium iodide (P3566)	• Propidium iodide (P3566)	• 7-AAD (A1310) • Propidium iodide (P3566)	• 7-AAD (A1310) • Propidium iodide (P3566)	• SYTOX® Red dye (S34859)	
		• DiI (V22885)			• DDAO-SE (C34553) • DiD (V22887)	
• Caltag™ Counting Beads (PCB-100) • CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)	• CountBright™ beads (C36950)

discrimination (Figure 3). Cells must be stained for viability in the absence of extraneous protein. These reagents are ideal for researchers who want to fix samples before analysis and also maintain dead-cell discrimination during intracellular staining.

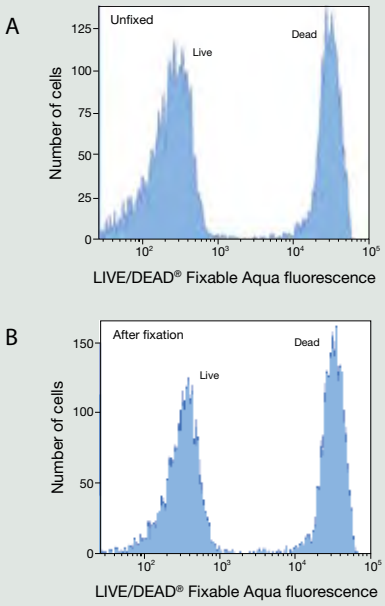


Figure 3—Use of fixable dead cell dyes. The reagents in the LIVE/DEAD® Fixable Aqua Stain Kit were used to differentially stain a mixture of live (left peak) and heat-treated Jurkat cells (right peak). The cells in **(A)** were not fixed; the cells in **(B)** were fixed in 3.7% formaldehyde following the staining reaction. Samples were analyzed by flow cytometry using 405 nm excitation and ~525 nm emission.

Indicators for metabolic, enzymatic, and other processes

Cells require metabolic and enzymatic functions to survive, and these activities are readily detected with a variety of fluorogenic reagents. General reductase activity can be measured with C_{12}-resazurin; esterase activity can be detected with calcein reagents (Figure 4), available for several excitation sources. These reagents are often paired with dead-cell dyes for better live/dead resolution. Although fluorescence is well retained, samples should be analyzed soon after staining. These reagents are not compatible with fixation protocols.

Monitoring mitochondrial membrane potential

Mitochondrial activity depends on an active membrane potential, which can change in response to a wide variety of environmental effects. Mitochondrial membrane potential dyes are sensitive indicators of cell stress.[1] These probes are generally positively charged molecules that sequester in the matrix of mitochondria that have active membrane potentials, causing a fluorescence increase or spectral shift. JC-1 is commonly used with apoptosis models. $DiIC_1(5)$ also allows membrane potential to be observed with red excitation, leaving 488 nm excitation available for other reagents (Figure 5). Due to the chemical properties of MitoTracker® Red dye, it becomes covalently linked to sulfhydryl moieties in active mitochondria, which in turn allows the stained sample to be fixed. JC-1, $DiOC_2(3)$, and $DiIC_1(5)$ are not compatible with fixation.

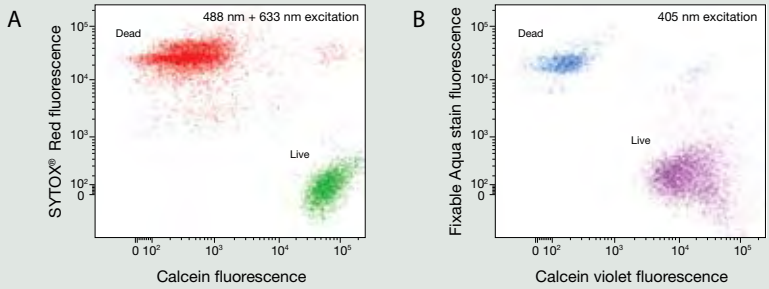


Figure 4—Combination of esterase substrates with dead-cell dyes using mixtures of heat-treated and untreated cells. (A) Jurkat cells were reacted with calcein, then stained with the impermeant DNA stain, SYTOX® Red dye, and analyzed by flow cytometry using 488 nm and 635 nm excitation. **(B)** Chinese hamster ovary (CHO) cells were stained according to the protocol in the LIVE/DEAD® Violet Viability/Vitality Kit (calcein violet and fixable violet dead-cell stain). Cells were analyzed by flow cytometry using 405 nm excitation.

Assessing cell proliferation

The proliferative state of a population of cells is an important parameter when studying live-cell function, particularly in cancer and drug-discovery research. Invitrogen offers several fluorescence-based kits and reagents for assessing cell proliferation (Table 1), including detection via nucleic acid stains, nucleoside incorporation, cell division, and overall cell counts.

Cell cycle profiles as a measure of proliferation

Detection of DNA content provides a snapshot of cells in a population that are in different stages of the cell cycle. Flow cytometry, in conjunction with modeling algorithms, provides a powerful tool to assess cells in G_0/G_1 phase versus S-phase, G_2, or polyploid.[2–4] DNA content is readily assessed in fixed cells with any of the impermeant DNA dyes, often requiring RNAse treatment. The DyeCycle™ dyes offer the ability to stain for DNA profile in live cells (Figure 6) with 405, 488 or 532 nm excitation. These dyes are generally used with a viability dye to exclude dead cells from the analysis. The dyes are not cytotoxic, allowing stained cells to be sorted and otherwise cultured or assessed with functional assays after staining.

Proliferation measured by nucleoside incorporation

Nucleoside incorporation provides direct measurement of new DNA synthesis. Traditionally, this has been performed by incorporating the nucleoside analog bromodeoxyuridine (BrdU) into DNA, followed by detection with an anti-BrdU antibody. Although effective, this method requires DNA denaturation (using HCl, heat, or DNase) to expose the BrdU to the antibody—a step that can be lengthy and difficult to perform consistently, and can adversely affect sample quality. The Click-iT™ EdU Cell Proliferation Assay eliminates the need to denature DNA, providing a superior alternative to the standard BrdU antibody-based method for measuring cell proliferation by flow cytometry. The Click-iT™ advantage is in the chemistry—small, unique, and bioorthogonal labeling and detection moieties that react very efficiently and specifically with one another. EdU (5-ethynyl-2′-deoxyuridine) is a nucleoside analog containing an alkyne. →

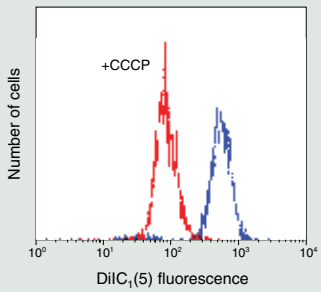


Figure 5—Decrease in $DiIC_1(5)$ fluorescence with the loss of mitochondrial membrane potential. Jurkat cells were stained with 50 nM $DiIC_1(5)$ alone (blue line) or in the presence of 50 µM CCCP (carbonyl cyanide 3-chlorophenylhydrazone, red line), used to disrupt mitochondrial membrane potential. Cells were analyzed by flow cytometry using 635 nm excitation and ~660 nm emission.

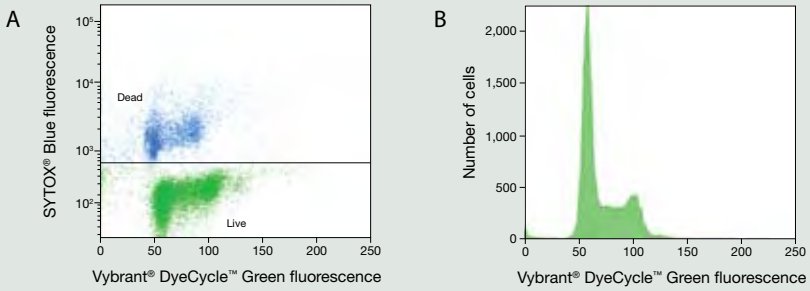


Figure 6—Viable cell gating with DyeCycle™ dyes. Jurkat cells from an overgrown culture were stained with Vybrant® DyeCycle™ Green stain, then stained with SYTOX® Blue dye and analyzed by flow cytometry using 488 nm and 405 nm excitation. The histogram **(B)** was gated on live cells **(A)** and shows DNA content distribution in live cells: G_0/G_1 and G_2/M phase histogram peaks are separated by the S-phase distribution. Inclusion of the dead cells would have given aberrant live-cell results.

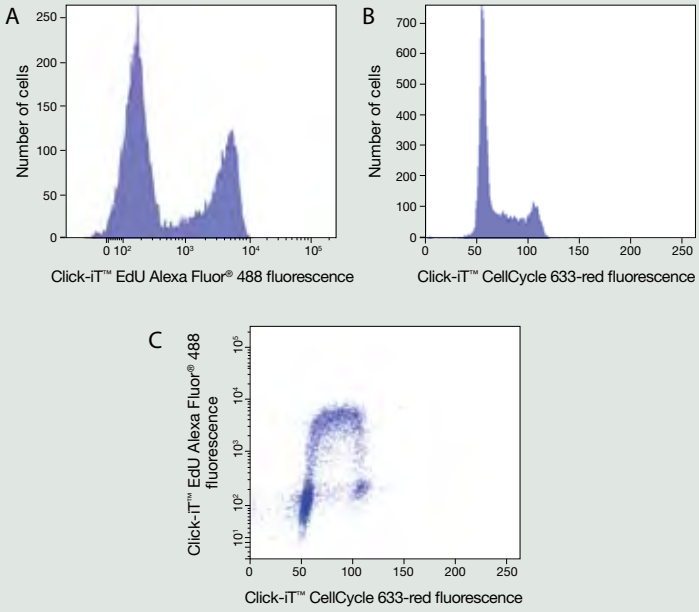


Figure 7—Cell proliferation using the Click-iT™ EdU Alexa Fluor® 488 Flow Cytometry Assay Kit. Jurkat cells were treated with 10 µM EdU for one hour and tested according to staining protocol. **(A)** Cell staining with Alexa Fluor® 488 azide using 488 nm excitation; clear separation of proliferating cells (which have incorporated EdU) and nonproliferating cells is demonstrated. **(B)** Cell staining with Click-iT™ CellCycle 633-red using a 633 nm excitation, showing DNA content distribution where G_0/G_1 and G_2/M phase histogram peaks are separated by the S-phase distribution. **(C)** Combination of the DNA content with the labeling of proliferating cells incorporating EdU; co-positive staining of cells provides the percentage of cells in S-phase (DNA synthesis).

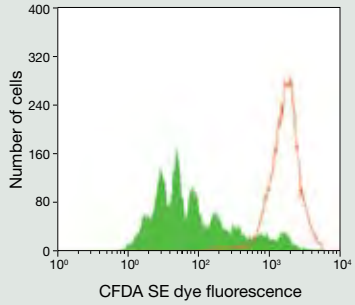


Figure 8—Human peripheral blood lymphocytes were harvested and stained with CFDA SE dye on Day 0. A portion of the population was arrested at the parent generation using mitomycin C (red peak). The rest of the sample was stimulated with phytohemagglutinin and allowed to proliferate for 5 days. Solid green peaks represent successive generations.

In a copper-catalyzed reaction, the alkyne reacts with a dye-labeled azide, forming a stable covalent bond. The small size of the azide reagents allows for efficient access to the DNA without the need for harsh cell treatment, thus simplifying the assay considerably, yet generating the same results (Figure 7). Click-iT™ EdU labeling is compatible with fixation protocols. For more details about Click-iT™ EdU, see pages 3–6.

New fixable dead-cell stains for your red laser

The LIVE/DEAD® Fixable Far Red and LIVE/DEAD® Fixable Near IR Dead Cell Stain Kits are some of the many Molecular Probes® products that Invitrogen has developed for red laser–equipped flow cytometers. The most up-to-date list can be found at **probes.invitrogen.com/products/flowcytometry**. Check back often to see what's new in flow cytometry tools.

Monitoring cell proliferation via generation analysis

Carboxyfluorescein diacetate, succinimidyl ester (5(6))-CFDA SE, also commonly called CFSE, spontaneously and irreversibly couples to cellular proteins by reaction with lysine side chains and other available amines. When cells divide, CFDA SE labeling is distributed equally between the daughter cells, and each successive generation in a population of proliferating cells is marked by a halving of cellular fluorescence intensity (Figure 8). Eight to ten successive generations have been identified this way.[5,6] It is also possible to perform multiplex analysis of CFDA SE and other markers to correlate cell division status with other cellular markers.[5,7,8]

To learn more about flow cytometry assays for assessing viability and vitality, visit **www.invitrogen.com/flowcytometry**. ■

References

1. Finkel, E. (2001) *Science* 292:624–626.
2. *Current Protocols in Cytometry*, 7.0.1–7.27.7 (2004).
3. *Practical Flow Cytometry*, 4th ed., Shapiro, H.M., ed. (2003).
4. Pozarowski, P. and Darzynkiewicz, Z. (2004) *Methods Mol Biol* 281:301–311.
5. Lyons, A.B. and Parish, C. R. (1994) *J Immunol Methods* 171:131–137.
6. Lyons, A.B. (1999) *Immunol Cell Biol* 77:509–515.
7. Fazekas de St. Groth, B. et al. (1999) *Immunol Cell Biol* 77:530–538.
8. Hodgkin, P.D. et al. (1996) *J Exp Med* 184:277–281.

Using Qdot® nanocrystal primary antibody conjugates in flow cytometry

TIPS FOR SAMPLE PREPARATION AND INSTRUMENT SETUP WHEN DETECTING SURFACE ANTIGENS.

Researchers are continually seeking to maximize the information that they receive from flow cytometry experiments by evaluating more labeled parameters in each sample. Qdot® nanocrystal conjugates are increasingly used in multispectral flow cytometry.[1–5] They provide a powerful way to multiply fluorophore selection using commonly available excitation sources. Invitrogen currently offers a growing selection of antibody conjugates using Qdot® 605, Qdot® 655, Qdot® 705, and Qdot® 800 nanocrystals. There are several unique advantages to using Qdot® nanocrystals, and here we will focus on their properties and use with common reagents and instrumentation in the flow cytometry workflow.

Qdot® nanocrystal technology is ideal for use in flow cytometry

Typical fluorescent dyes have excitation and emission spectra with relatively small Stokes shifts, which means that the optimal excitation wavelength is close to the emission peak. Qdot® nanocrystals have broad absorption spectra that increase dramatically at shorter excitation wavelengths. Their emission peaks are narrow and symmetrical and do not change with variations in the excitation source (Figure 1). Qdot® nanocrystals are desirable in that they require minimal single-laser compensation when using a single excitation source. Qdot® nanocrystals are optimally excited by a UV or violet (405–407 nm) laser, although sufficient excitation can also be obtained with other sources as discussed below.

The use of nanocrystal conjugates allows the addition of one to six colors, all excited from the violet laser, to panels using existing organic dyes. Qdot® nanocrystals provide the additional advantages of brightness and photostability.

Using Qdot® nanocrystal conjugates for surface antigens

Qdot® nanocrystal conjugates may be used in the same way as conventional conjugates. Conjugates are provided at a specific concentration of Qdot® nanocrystal, usually 1–2 µM, and this concentration can be used to standardize experiments. Because staining conditions may vary, reagents should be titered with samples to obtain optimal staining concentrations. Figure 2 shows typical staining patterns for a number of Qdot® antibody conjugates.

Reagents for sample preparation

Most conventional reagents used for erythrocyte lysis, including ammonium chloride and Cal-Lyse™ reagent, have minimal effects on the fluorescence intensity of cells stained with Qdot® conjugates (Figure 3). FACS™ Lysing Solution (BD Biosciences) usually has minimal impact on Qdot® nanocrystal fluorescence, although we have reports of occasional decreases in Qdot® nanocrystal fluorescence that may be related to particular batches of FACS™ Lysing Solution. In some cases, a decrease in fluorescence can be related to fixatives present in a lysis reagent such as BD™ PhosFlow Lyse (BD Biosciences), which may alter the antigenic determinants recognized by particular antibodies. →

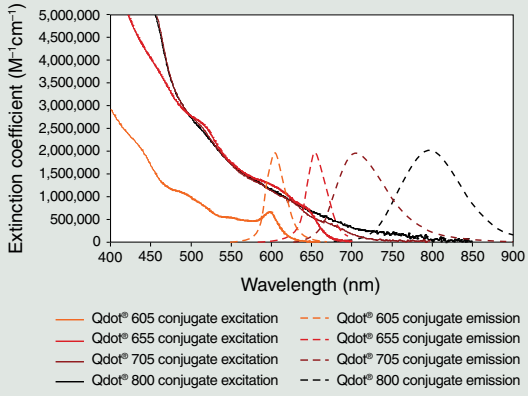


Figure 1—Extinction coefficient and emission profiles for selected Qdot® nanocrystals. Excitation is presented as extinction coefficient (left axis); emission is normalized to maximum peak height.

Table 1—BD™ LSR II filter combinations to detect selected Qdot® nanocrystals.

Fluorophore	Em*	Configuration A: Narrow bandpass filters		Configuration B: Broad bandpass filters[1,2]	
Qdot® 605 nanocrystal	605	570LP	605/20 nm	595LP	605/40 nm
Qdot® 655 nanocrystal	655	640LP	655/20 nm	640LP	660/40 nm
Qdot® 705 nanocrystal	705	690LP	720/20 nm	670LP	705/70 nm
Qdot® 800 nanocrystal	800	750LP	780/60 nm	750LP	780/60 nm

* Emission maximum, in nm.

Aldehyde-based fixatives may cause a small change in Qdot® nanocrystal fluorescence. For example, Figure 3 shows a 2-fold reduction in fluorescence after fixation with formaldehyde, although negative peak fluorescence also decreased. This change in fluorescence is generally tolerable given the population resolution achieved with Qdot® conjugates.

Reagents commonly used to permeabilize cells after fixation have not been shown to adversely affect Qdot® conjugate fluorescence. Reagents tested include FIX & PERM® Reagent B, BD Cytofix/Cytoperm™ reagent (BD Biosciences), 0.1% saponin, 0.05% Triton X®-100, and methanol solutions (Figure 3).

Instrument setup and filter selection

Qdot® nanocrystals exhibit the brightest emission when excited with either a UV or a violet laser source, but acceptable fluorescence can be obtained from any excitation below the emission maximum of a given nanocrystal. Therefore, samples stained with Qdot® nanocrystal conjugates can be analyzed on any cytometer that has an appropriate filter selection.

Because most nanocrystals have symmetrical and relatively narrow emission peaks (Figure 1), emission can be efficiently detected with a 20 nm wide filter centered on the emission maximum of a given nanocrystal. Users can minimize the need to correct for spectral overlap between Qdot® nanocrystals by selecting reagents with at least a 40 nm separation between maximum emissions. Table 1 shows two filter schemes that can be used with Qdot® nanocrystals on a BD™ LSR II cytometer. Configuration A uses narrow bandwidth filters to minimize effects of spectral overlap. Configuration B, developed in the laboratory of Mario Roederer, uses wider bandpass filters to collect more photons, and longpass filters close the emission maximum to minimize spectral overlap.

Nanocrystals can also be used efficiently on instruments that have 488 nm excitation sources. For instruments with fixed filter configurations, such as the BD™ FACScan™ cytometer (BD Biosciences), you can match specific nanocrystals to the filters installed on the instrument.

Extend your color palette with Qdot® nanocrystals

Qdot® nanocrystal conjugates of monoclonal antibodies are powerful and easy-to-use tools to extend the number of colors in your multicolor flow cytometry panels. They are compatible with standard sample preparation reagents and staining protocols. They can be used efficiently on cytometers with UV or violet excitation sources, and with selection of appropriate filters. As with other fluorescent conjugates in multicolor work, care must be taken in designing a reagent panel to minimize spectral overlap, with particular attention to the cross-laser excitation of nanocrystals. Nanocrystals can also be used efficiently on cytometers with 488 nm or longer excitation if the nanocrystals are matched to available emission filters. For more information, visit **www.invitrogen.com/qdotinflow**. ■

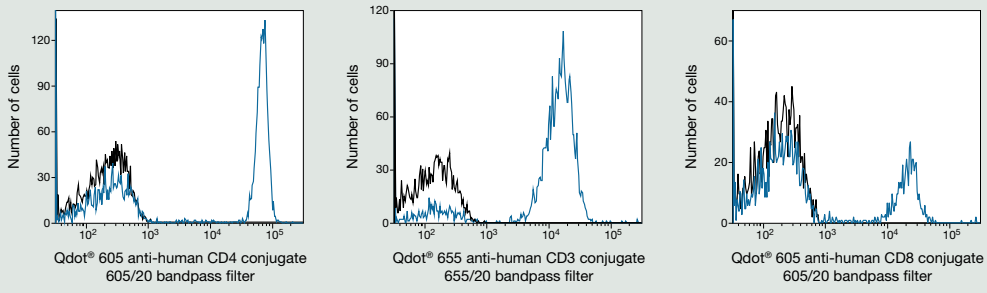


Figure 2—Staining profiles for Qdot® nanocrystal-conjugated antibodies. Human peripheral blood lymphocytes were stained with the specified antibody–Qdot® conjugates. Samples were analyzed using a BD™ LSR II cytometer (BD Biosciences) with 405 nm excitation and the specified emission filters. The blue peaks correspond to stained lymphocytes, and the black peaks show the position of unstained cells in the histograms.

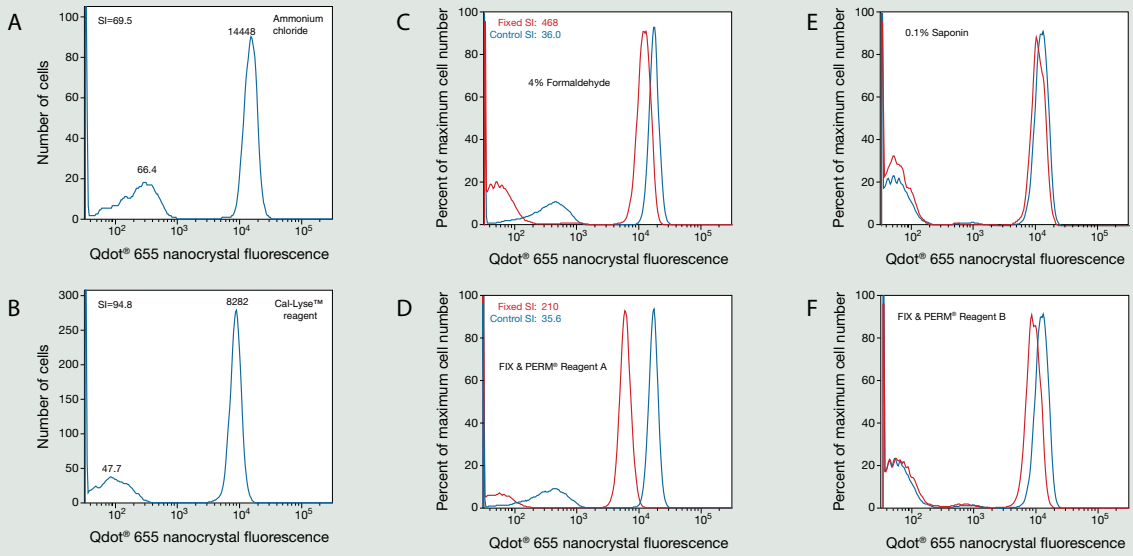


Figure 3—Representative results using common sample preparation reagents. Human peripheral blood lymphocytes were stained with mouse anti–human CD4, Qdot® 655 conjugate, before treatment with the specified reagents: erythrocyte lysis **(A, B)**, fixatives **(C, D)** and permeabilizing reagents after treatment with formaldehyde **(E, F)**. Samples were analyzed using a BD™ LSR II cytometer (BD Biosciences) with 405 nm excitation and a 655/20 nm emission filter. Histograms are smoothed and labeled with geometric mean fluorescence values. Staining index (SI), to quantify population resolution, is calculated as the difference in population mean fluorescence values divided by twice the negative peak standard deviation.

References

1. Chattopadhyay, P.K. et al. (2006) *Nat Med* 12:972–977.
2. Perfetto, S.P. et al. (2004) *Nat Rev Immunol* 4:648–655.
3. Telford, W.G. (2004) *Cytometry A* 61:9–17.
4. Chattopadhyay, P.K. et al. (2007) in *Quantum Dots, Applications in Biology*. M.P. Bruchez and C.Z. Hotz, ed. Humana Press, Totowa, NJ. pp. 175–184.
5. Abrams, B. and Dubrovsky, T. (2007) in *Quantum Dots, Applications in Biology*. Bruchez, M.P. and Hotz, C.Z., ed. Humana Press, Totowa, NJ. pp. 185–206.

FIX & PERM® is a trademark of An Der Grub Bio Research GmbH.

Product	Quantity	Cat. no.
CD3, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10054
CD3, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10012
CD4, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10008
CD4, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10007
CD4, mouse anti-human, Qdot® 800 conjugate	100 µl	Q10064
CD8, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10009
CD8, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10055
CD8, mouse anti-human, Qdot® 705 conjugate	100 µl	Q10059
CD14, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10013
CD14, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10056
CD14, mouse anti-human, Qdot® 800 conjugate	100 µl	Q10064
CD27, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10065
CD27, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10066
CD38, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10053
CD38, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10057
CD45, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10051
CD45, mouse anti-human, Qdot® 705 conjugate	100 µl	Q10062

Product	Quantity	Cat. no.
CD45RA, mouse anti-human, Qdot® 605 conjugate	100 µl	Q10047
CD45RA, mouse anti-human, Qdot® 655 conjugate	100 µl	Q10069
Mouse IgG2a, Qdot® 605 conjugate	100 µl	Q10014
Mouse IgG2a, Qdot® 655 conjugate	100 µl	Q10015
Mouse IgG2a, Qdot® 705 conjugate	100 µl	Q10076
Mouse IgG2a, Qdot® 800 conjugate	100 µl	Q10075
Mouse IgG2b, Qdot® 605 conjugate	100 µl	Q10074
Mouse IgG1, Qdot® 605 conjugate	100 µl	Q10073
Cal-Lyse™ Whole Blood Lysing Solution	25 ml	GAS-010
Cal-Lyse™ Whole Blood Lysing Solution	100 ml	GAS-010S-100
High-Yield Lyse	500 ml	HYL-250
FIX & PERM® Reagent A	5 ml	GAS001S-5
FIX & PERM® Reagent A	100 ml	GAS001S-100
FIX & PERM® Reagent B	5 ml	GAS002S-5
FIX & PERM® Reagent B	100 ml	GAS002S-100
FIX & PERM® Reagents	50 ml	GAS003
FIX & PERM® Reagents	200 ml	GAS004

Omnia® kinase assay for cell lysates

REAL-TIME KINETIC ACTIVITY MEASUREMENTS IN UNDER AN HOUR.

For years, radioactive filter binding assays have been widely used to directly measure kinase phosphorylation of target proteins. This format, however, does not meet the current demand for assays with higher throughput capabilities and greater ease of use. Omnia® kinase assays offer the reliability of radioactive filter binding assays, but with some big advantages. These assays provide an easy way to measure the activity of a wide range of serine/threonine (Ser/Thr) and tyrosine (Tyr) kinase proteins in real time. Forget about running multiple endpoint experiments—Omnia® kinase assays give you accurate, minute-by-minute activity data for a specific kinase. In addition, you avoid the complications of hazardous material handling and disposal.

Omnia® kinase assay technology

The Omnia® kinase assay uses peptide substrates that contain a chelation-enhanced fluorophore called Sox. This biosensor provides a direct fluorescence-based measurement of kinase activity. Upon phosphorylation, Mg^{2+} is chelated between the added phosphate group and the Sox moiety, forming a complex that causes an increase in fluorescence from the Sox residue (Figure 1). The fluorescence signal is directly proportional to the amount of peptide phosphorylation and can be monitored in kinetic mode.

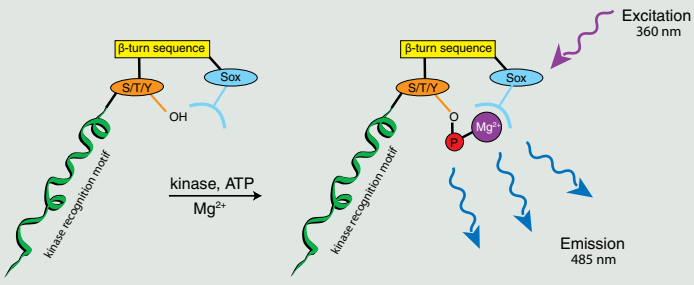


Figure 1—Binding of Mg^{2+} and the Sox moiety results in increased fluorescence. Upon phosphorylation, Mg^{2+} is chelated to form a bridge between the Sox moiety and the phosphate group, resulting in an increase in fluorescence (excitation at 360 nm and emission at 485 nm).

Measure kinase activity in cell lysates

Kinase activity measurements in crude cell lysates provide a better understanding of kinase behavior in a complex environment. Furthermore, characterization of kinases in cell lysates may be a critical step to qualify biochemical leads before performing translational research in animal models. To meet the increasing demand for assays to determine kinase activity in cell lysates, Invitrogen has developed three Omnia® kinase assay formats designed specifically for these samples (Table 1).

Omnia® Lysate Assays use peptide substrates that are inherently highly selective for specific kinases. To enhance selectivity, most assays include the addition of off-target inhibitors, providing the convenience of a homogeneous assay. Omnia® Agarose Bead IP Assays and Omnia® Plate IP Assays require an immunoprecipitation (IP) step to first isolate the kinase of interest from the complex mixture of proteins before performing the kinase assay (Figure 2). This method provides great specificity (Figure 3). The specific antibodies are either provided with

Table 1—Advantages of each Omnia® kinase assay format.

Format	Description	Advantages
Omnia® Lysate	Crude cell or tissue lysate	Homogeneous assay—no-wash step
Omnia® Agarose Bead IP	Antibody-coated, bead-based capture	Flexibility to vary the number of beads per reaction
Omnia® Plate IP	Antibody-coated, plate-based capture	Easy to use in 96-well format and increased sensitivity for cascade assays

agarose beads or coated onto a 96-well plate. All Agarose Bead IP Kits measure direct kinase phosphorylation of the substrate, whereas all Plate IP kits, with the exception of the Syk kit, offer indirect measurements of the kinase. Indirect cascade reactions offer greater flexibility for studying signaling events. Cascade reactions amplify the signal and enable inhibition studies of select kinases in the cascade. Furthermore, Plate IP assays offer better sensitivity and reduce the cell lysate sample size and assay duration.

Accurate results in real time

Invitrogen offers Omnia® substrates that have been validated for more than 190 kinases. Simply perform an immunoprecipitation of your particular kinase from the cell lysate, then run the Omnia® kinase assay. Find out how easy measuring kinase activity can be at www.invitrogen.com/omnia. ■

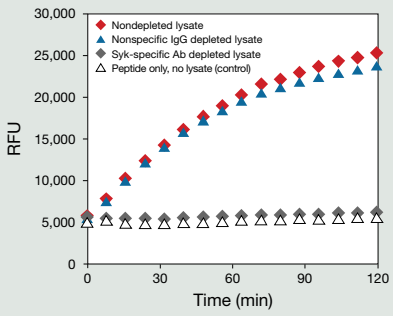


Figure 3—High selectivity of the Omnia® Agarose Bead IP Kit. Anti-IgM antibody–stimulated RAMOS cell lysates were tested for Syk kinase activity using the Omnia® Agarose Bead IP Kinase Assay Kit for Syk (Cat. no. KNZ6081). Kinase activity was significantly reduced in Syk-specific antibody-depleted lysate compared to anti-IgM or nonspecific IgG-depleted lysate, demonstrating specificity for Syk.

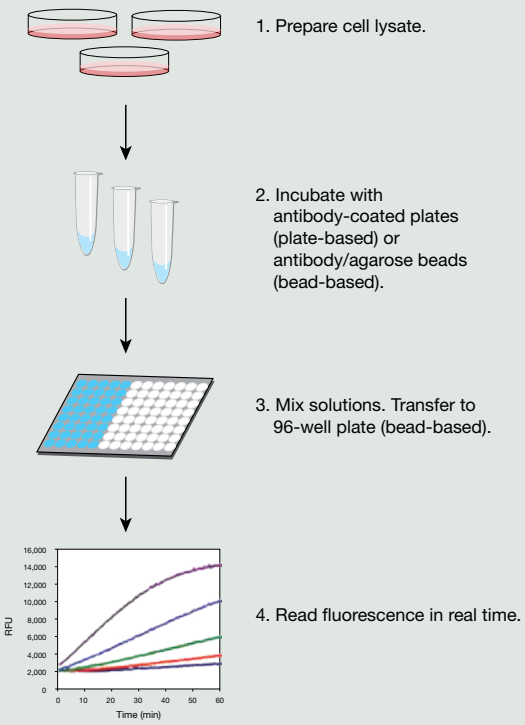


Figure 2—The Omnia® IP kinase assay procotol.

Product	Quantity	Cat. no.
Crude Lysate Kits		
Akt	100 × 50 µl assays	KNZ0011
PKA	100 × 50 µl assays	KNZ0021
MAPKAP-K2	100 × 50 µl assays	KNZ0031
Syk	100 rxns	KNZ0041
Agarose Bead IP Kits		
Akt1	40 rxns	KNZ6011
Akt3	40 rxns	KNZ6021
ERK1/2	40 rxns	KNZ6031
MAPKAP-K2	40 rxns	KNZ6041
p70-S6K	40 rxns	KNZ6051
RSK	40 rxns	KNZ6061
c-Src	40 rxns	KNZ6071
Syk	40 rxns	KNZ6081
Plate IP Kits		
B-Raf	96 rxns	KNZ7011
MEK1	96 rxns	KNZ7031
ERK1/2	96 rxns	KNZ7021
p38 MAPK	96 rxns	KNZ7041
Syk	96 rxns	KNZ7051

A no-wash indicator for high-throughput calcium detection

H1 HISTAMINE GPCR SCREENING USING PREMO™ CAMELEON CALCIUM SENSOR.

Second messengers such as cAMP and Ca^{2+} are key components of cell signaling pathways. Monitoring these transient second messengers in their native states requires an easy-to-use, effective tool for high-throughput screening (HTS). The combination of a fluorescent protein biosensor and efficient BacMam delivery technology [1] provides an excellent method for expressing genetically encoded sensors in mammalian cells. Using this approach, we screened a H1 histamine G protein–coupled receptor (GPCR) stably expressed in HEK 293T cells and demonstrated the utility of Premo™ Cameleon Calcium Sensor for high-throughput calcium flux measurements.

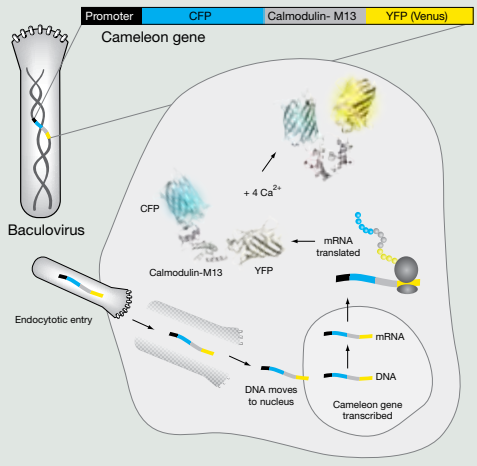


Figure 1—Schematic representation of BacMam-mediated transduction and expression of the Premo™ Cameleon Calcium Sensor. The combination of fluorescent protein biosensors and BacMam delivery technology yields easy-to-use, genetically encoded sensors for cell-based assays. A BacMam virus carrying the cameleon fusion gene (blue-gray-yellow) transduces a cell and traffics to the nucleus where only the cameleon gene is transcribed. Baculovirus promoters are not recognized by the mammalian transcriptional machinery—hence no virus replication and no toxicity. Following transcription, the cameleon mRNA is expressed in the cytosol—or a specific compartment—depending on the presence of targeting tags.

A powerful combination for cell-based assays

Premo™ Cameleon Calcium Sensor is based on the CFP:YFP fluorescence resonance energy transfer (FRET) pair linked by a calcium-responsive calmodulin-M13 unit.[2] The gene construct is carried on a baculovirus chromosome, which efficiently delivers its genetic payload to mammalian cells. Because only the transgene cassette with a mammalian promoter (here CMV) is recognized by the mammalian cell machinery, only the Premo™ Cameleon Calcium Sensor is expressed (Figure 1).

The BacMam advantage

BacMam-mediated transduction is efficient and reproducible in a large number of cell types without apparent cytopathic effects. Since its introduction 15 years ago, BacMam technology has been used in more than 70 cell types, including primary and stem cells. Table 1 lists examples of cell lines that are efficiently transduced by BacMam technology. Protein expression is transient in the absence of selection pressure and can

Table 1—Typical transduction efficiencies obtained using the standard Premo™ Cameleon Calcium Sensor protocol.

Cell line	Transduction efficiency
CHO, CHO K1, CHO M1WT3, etc.	50%–80%
COS-7	>90%
CRL-1973 (NTERA-2)	30%
HEK 293	>90%
HeLa	60%–70%
HepG2	>90%
Indian muntjac	80%–90%
Adult porcine heart stem cells	40%–50%
NIH 3T3	40%–50%
OMK	50%–60%
Primary cardiac smooth muscle cells	40%–50%
Spiral ganglial neurons (rat)	50%–75%
SK-BR-3	40%–50%
U-2 OS	>90%
WI38	30%–40%
Cells of hematopoietic lineages	Not readily amenable

therefore easily be adjusted to a desired level. This is useful for work with difficult proteins such as many drug targets (e.g., ion channels and nuclear receptors), where sustained levels can be difficult to achieve in stably transduced cells.[3,4] The use of BacMam technology in HTS applications has recently been reviewed;[3] additional advantages include user safety; consistent expression within and between plates; adaptability to a number of assay formats; and compatibility with automated liquid handling. For a further discussion of BacMam technology advantages, see pages 8–11.

In addition, the efficient BacMam delivery and the genetic content of the Premo™ Cameleon Calcium Sensor enable transduction of large quantities of cells in batch mode. These cells can be stored as frozen aliquots and used as needed; this approximates the consistency of stable cell lines at a fraction of the time and cost, and without the risk of genetic drift. Expression of the fluorescent protein is unaffected by freezing and remains stable for more than 120 hours, depending on cell line; following thawing and plating, cells can be used within hours and multiple screens can be performed. There is no need to replace the medium before adding additional compounds. Premo™ Cameleon Calcium Sensor improves the HTS workflow by decoupling dye loading from calcium mobilization.

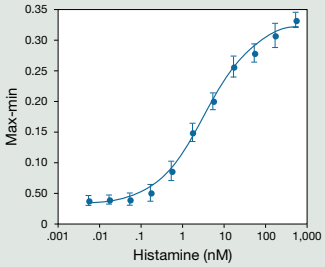


Figure 3—Histamine response of HEK 293T cells. HEK 293T cells stably expressing H1 histamine GPCR were transduced with Premo™ Cameleon Calcium Sensor. Histamine response was measured on a Hamamatsu FDSS6000 in 384-well plates. The y-axis is a measure of the YFP:CFP ratio after calcium mobilization (max) minus the YFP:CFP ratio before histamine addition (min). Standard deviation error bars are displayed for the 16 replicates that make up each data point.

Simple workflow for screening applications

Typically, cells are transduced one day before screening. The prepackaged Premo™ Cameleon Calcium Sensor constructs are ready for immediate use on Day 1, containing all components (the virus and the cameleon gene) required for cellular delivery and expression. There is no need for cloning or plasmid purification, lipids, dye-loading chemicals, or other potentially harmful treatments. Simply add Premo™ Cameleon Calcium Sensor to plated cells and incubate 2–4 hours (primary and stem cells require shorter incubation times); after removing the sensor, add Premo™ enhancer and incubate 1.5–2 hours, then remove the enhancer, add growth medium, and incubate overnight. On Day 2, screening can be conducted immediately in complete medium, without any wash steps. The typical screening workflow is shown in Figure 2.

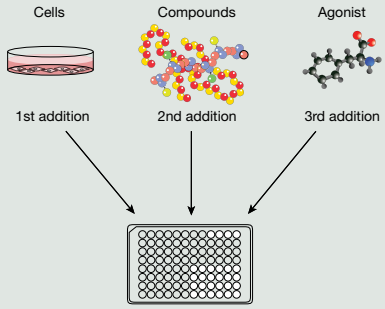


Figure 2—Typical screening workflow using Premo™ Cameleon Calcium Sensor and other BacMam sensors. Cells are seeded, then compounds are added in full medium for a true no-wash calcium assay (no dye loading required). An agonist can be added to the wells as an optional third step.

Validated for screening applications

To validate Premo™ Cameleon Calcium Sensor for screening applications, we first confirmed that the reagent showed standard pharmacology and backwards compatibility to traditional calcium dyes, such as Fluo-4. Both criteria were confirmed in a CHO-M1 cell model by inducing calcium flux with carbachol, giving IC_{50} values of 21 nM and 28 nM for Premo™ Cameleon Calcium Sensor and Fluo-4, respectively. Because Premo™ Cameleon Calcium Sensor is a ratiometric indicator, the signal should be independent of cell number within the range of sensitivity; this was demonstrated at four cell concentrations (5,000–30,000) in a 96-well plate (data not shown).

HTS compatibility

To demonstrate the utility of Premo™ Cameleon Calcium Sensor in HTS, we screened an H1 histamine G protein–coupled receptor stably expressed in HEK 293T cells against the LOPAC^{1280}™ library (Sigma-Aldrich Biotechnology LP) using a Hamamatsu FDSS6000. The screen was performed in agonist and antagonist modes on the same 384-well plates in true no-wash fashion by first adding cells in complete media, then compounds, and finally a known agonist (5 nM histamine) to determine potential antagonists. The ratiometric nature of Premo™ Cameleon Calcium Sensor led to Z′ values that averaged 0.81 and a Z score of 0.61 (Figure 3). →

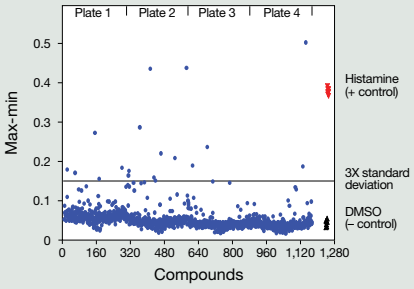


Plate	Z′	Z factor
1	0.77	0.62
2	0.81	0.62
3	0.86	0.64
4	0.80	0.54

Figure 4—H1 HEK 293T cells transduced with Premo™ Cameleon Calcium Sensor were screened in agonist mode against the LOPAC^{1280}™. Day 1: Cells were batch transduced with cameleon. Day 2: Cells were trypsinized and plated in 384-well plates in complete medium with serum. Day 3: The calcium mobilization assay was performed in the Hamamatsu FDSS6000 in 384-well plates. Compounds were added to the cells 5 seconds after establishing a baseline. Kinetic reads lasted 3 minutes. A line was drawn at 3 times the standard deviation of the mean of the compounds to denote "hits." Multipoint (n = 8) dose-response curves were generated to establish retest rates for the top 16 compounds showing agonist activity (see Figure 5). Z′ factors are shown for the data points on the top half of the curve relative to a "no histamine/DMSO" control.

The same 384-well plates of H1 HEK 293T cells used in the agonist screen were used to collect potential antagonist "hits." Approximately 25 minutes after LOPAC^{1280}™ compounds were added to the plates, an EC_{80} value (5 nM) of a known agonist (histamine) was added to all wells of the plate (Figure 4). Single-point dose-response curves were generated for the top 16 compounds showing potential antagonist activity (Figure 5).

Making the move to large-scale screening

With a residence time of several days and Z′ values greater than 0.5, Premo™ Cameleon Calcium Sensor is ideal for use in HTS. Unlike other indicators, this no-wash ratiometric calcium sensor does not require dye loading or the use of suppression dyes or multidrug resistance transporter inhibitors such as probenecid for cellular retention. The H1 histamine GPCR screen described here using cells transduced with Premo™ Cameleon Calcium Sensor illustrates the utility and efficiency of this reagent in HTS mode. To learn more about Premo™ Cameleon Calcium Sensor with BacMam delivery technology, visit **www.invitrogen.com/premo**. ■

References

1. Kost, T.A. (2005) *Nat Biotech* 23:567–575.
2. Nagai, T. (2004) *Proc Natl Acad Sci U S A* 101:10554–10559.
3. Kost, T.A. et al. (2007) *Drug Disc Today* 12:396–403.
4. Condreay, J.P. et al. (2006) *Adv Virus Res* 68:255–286.

Product	Quantity	Cat. no.
Premo™ Cameleon Calcium Sensor, for 10 microplates	1 kit	P36207
Premo™ Cameleon Calcium Sensor, for 100 microplates	1 kit	P36208

Note: No special license is required to use Premo™ Cameleon Calcium Sensor.

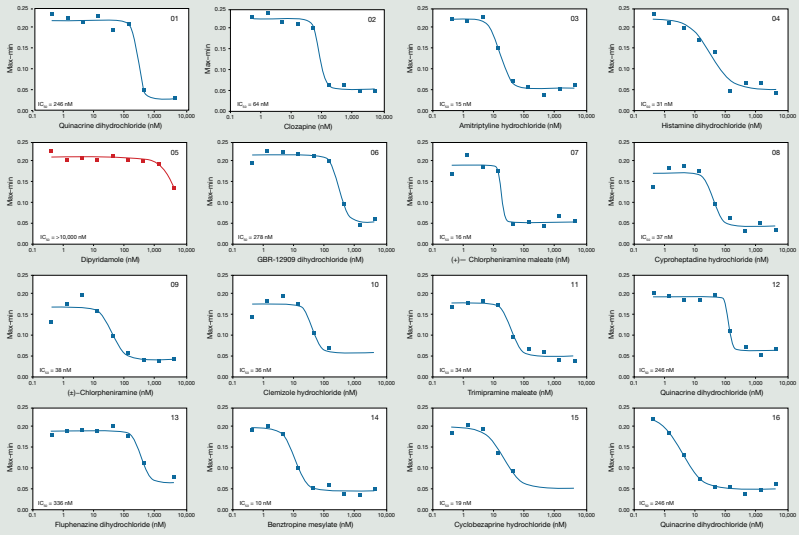


Figure 5—Dose-response curves of the 16 hits identified in an antagonist screen of H1 HEK 293T cells transduced with Premo™ Cameleon Calcium Sensor. One of the 16 hits appeared to be a false positive (shown in red).

Getting a head start on Alzheimer's disease detection

Li, G., Sokal, I., Quinn, J.F., Leverenz, J.B., Brodey, M., Schellenberg, G.D., Kaye, J.A., Raskind, M.A., Zhang, J., Peskind, E.R., and Montine, T.J. (2007) CSF tau/$A\beta_{42}$ ratio for increased risk of mild cognitive impairment: a follow-up study. *Neurology* 69:631–639.

Are there reliable biomarkers for the earliest, asymptomatic stage of Alzheimer's disease? The neurological damage associated with Alzheimer's disease (AD) is believed to begin many years before the onset of the minor cognitive impairment (MCI) that often serves as the first warning sign of the disease. As new therapeutic strategies are developed that show promise for slowing the course of Alzheimer's, there is an increasing need to be able to identify individuals in this earliest, essentially asymptomatic, latent phase of the disease. Previous studies have identified two proteins in cerebrospinal fluid (CSF)—tau and $A\beta_{42}$—whose levels change predictably in patients exhibiting AD dementia, with tau levels increasing and $A\beta_{42}$ levels decreasing relative to age-matched controls. The tau/$A\beta_{42}$ ratio has previously been used to assess the likelihood that individuals with MCI will progress to dementia.

In the current study, the authors attempt to establish cutoff values for the tau/$A\beta_{42}$ ratio that characterize the transition from latent Alzheimer's disease to this intermediate MCI stage. They analyzed tau and $A\beta_{42}$ levels in CSF from donor patients using a BioSource™ Luminex® technology–based assay system. Through statistical analysis of the data, they were able to define significant, age-independent values for the tau/$A\beta_{42}$ ratio that accurately characterized their patient population into "normal", "high", "MCI", "AD", and "other" subgroups. The authors hypothesized that a number of the individuals in the high CSF tau/$A\beta_{42}$ ratio subgroup may have latent Alzheimer's, and sought to correlate their data with the frequencies of the ε4 allele of the APOE gene, a known genetic marker for AD. Their analysis showed an elevated frequency of APOE ε4 in the high tau/$A\beta_{42}$ group, consistent with the supposition that these individuals were at a greater risk of progressing into MCI and eventual AD dementia. The authors suggest that such analysis could form the basis of clinical methodologies for the early identification of increased Alzheimer's risk in cognitively normal adults, allowing for more effective application of therapies to suppress Alzheimer's disease–related neurological damage. ■

Product	Quantity	Cat. no.
Aβ40	100 tests	LHB3481
Aβ42	100 tests	LHB3441
Aggregated Aβ (includes buffer kit, cannot be multiplexed)	100 tests	LHB3491
Aggregated α-synuclein (includes buffer kit, cannot be multiplexed)	100 tests	LHB0071
BDNF * (validated for serum, plasma, and CSF)	100 tests	LHC7071
GDNF * (validated for serum, plasma, and CSF)	100 tests	LHC7041
PDGF-BB	100 tests	LHG0041
Tau (total)	100 tests	LHB0041
Tau [pS199]	100 tests	LHB7041
Tau [pT181]	100 tests	LHB7051

* Compatible with the Neuroscience Buffer Kit (LNB0001).

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Product	Quantity	Cat. no.
Click-iT™ AHA (L-azidohomoalanine), for nascent protein synthesis	5 mg	C10102
Click-iT™ Tetramethylrhodamine (TAMRA) Protein Analysis Detection Kit, UV/532 nm excitation	10 rxns	C33370
Click-iT™ Dapoxyl® Protein Analysis Detection Kit, for UV excitation	10 rxns	C33371
Click-iT™ Biotin Protein Analysis Detection Kit	10 rxns	C33372

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Product	Quantity	Cat. no.
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Quant-iT™ RNA BR Assay Kit	100 assays	Q10210
	500 assays	Q10211

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Product	Quantity	Cat. no.
Mercator™ Phospho-Akt Pathway Array (13-plex)	1 slide / 208 tests	BHM9041
Mercator™ Phospho-Akt Pathway Array (13-plex)	4 slides / 832 tests	BHM9044

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Product	Application	Quantity	Cat. no.
anti-GFP, rabbit IgG fraction, biotin-XX conjugate	ICC, WB	100 µl	A10259
anti-GFP, rabbit IgG fraction, HRP conjugate	ICC, WB	200 µg	A10260
anti-GFP, chicken IgY fraction	ICC, WB	100 µl	A10262
anti-GFP, chicken IgY, fraction, biotin-XX conjugate	ICC, WB	100 µl	A10263
anti-GFP, mouse IgG2a, monoclonal 3E6	IP, IHC	100 µg	A11120
anti-GFP, mouse IgG1, monoclonal 11E5	WB	100 µg	A11121

ICC=immunocytochemistry, WB=western blot, IP=immnoprecipitation, IHC=immunohistochemistry.

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EGFR Total Antibody Bead Kit	100 tests	LHR9061
Human c-Kit Total AB Bead Kit	100 tests	LHO0391
Human c-Met Total AB Bead Kit	100 tests	LHO0251

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Human IL-21 ELISA Kit	192 tests	KHC0212
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Mouse Leptin ELISA	192 tests	KMC2282

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IGF-1 (Hu)	100 µg*	PHG9071

* Other sizes available; visit www.invitrogen.com/proteins to see our complete list.

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Target protein	Clonality, clone (isotype)	Reactive species	Applications	Cat. no.
beta-Catenin [npaa 27–37]	β-cat-8E4 (Ms IgG1)	Hu, Ms, Rt	WB, E, ICC, IHC (FFPE)	44207M
beta-Catenin [npaa 35–50]	β-cat-7A7 (Ms IgG1)	Hu, Ms, Rt	WB, E, ICC, IHC (FFPE)	44206M
beta-Catenin [pY86]	β-cat-24E1 (Ms IgG1)	Hu, Ms, Rt	WB, E	44210M
beta-gamma-Catenein [pY654/pY643]	1B11 (Ms IgG1)	Cn, Hu, Ms, Rt	WB	44212M

Ms=mouse, Rb=rabbit, Hu=human, Rt=rat, Cn=canine; WB=western blot, E=ELISA, ICC=immunocytochemistry, IHC (FFPE)=immunohistochemistry (formalin-fixed, paraffin-embedded).

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Recently published

A LOOK AT HOW YOUR FELLOW RESEARCHERS ARE USING INVITROGEN™ PRODUCTS.

Labeling-induced perturbation of antibody–antigen binding interactions. Although it is logical to expect that labeling of antibodies would produce changes in their antigen binding behavior, detailed physical investigations of these effects are relatively rare. Blake and coworkers describe such a study in the case of a monoclonal antibody against uranium chelates. They report that labeling via amine reactive chemistry with either Alexa Fluor® 488 carboxylic acid, succinimidyl ester, or Cy®5 succinimidyl ester (GE Healthcare UK Limited) induces positive co-operativity in the antibody–antigen interaction. In contrast, non-covalent labeling with the Zenon® Alexa Fluor® 647 mouse IgG1 labeling reagent results in no cooperativity and a 3–13 fold increase in binding affinity relative to the unlabeled antibody.

Blake, R.C., 2nd, Li, X., Yu, H., Blake, D.A. (2007) Covalent and noncovalent modifications induce allosteric binding behavior in a monoclonal antibody. *Biochemistry* 46:1573–1586.

Reference cell line characterization for human embryonic stem cell (hESC) research. A team including researchers from Invitrogen's Corporate Research Laboratories describe characterization of human embryonal carcinoma (EC) line 2102Ep as a prospective reference standard to which other hESC lines can be compared. Among many tools from Invitrogen's stem cell analysis portfolio, they used Alexa Fluor® 488 dye–labeled secondary antibodies for flow cytometric immunophenotyping and the ELF® 97 Endogenous Phosphatase Detection Kit for endogenous alkaline phosphatase activity measurements. MicroRNAs (miRNAs) were isolated from total RNA using the PureLink™ miRNA Isolation Kit and labeled using the NCode™ direct labeling system for microarray analysis of abundance and differential expression.

Josephson, R., Ording, C.J., Liu, Y., Shin, S., Lakshmipathy, U., Toumadje, A., Love, B., Chesnut, J.D., Andrews, P.W., Rao, M.S., Auerbach, J.M. (2007) Qualification of embryonal carcinoma 2102Ep as a reference for human embryonic stem cell research. *Stem Cells* 25:437–446.

PicoGreen® imaging of mitochondrial DNA (mtDNA) depletion. In addition to its preeminent application for DNA quantitation in solution, PicoGreen® dsDNA Quantitation Reagent is also an enabling tool for image-based analysis of mitochondrial DNA (mtDNA) in living cells. Morphology and colocalization with organelle-specific stains such as MitoTracker® Deep Red FM provide clear discrimination of mtDNA from nuclear DNA. Saffran and coworkers describe the use of this combination of reagents to elucidate the role of the viral UL12 gene product and its isoforms in mtDNA depletion induced by herpes simplex virus (HSV) infection of HeLa cells.

Saffran, H.A., Pare, J.M., Corcoran, J.A., Weller, S.K., Smiley, J.R. (2007) Herpes simplex virus eliminates host mitochondrial DNA. *EMBO Rep* 8:188–193.

Novel probes for adenosine A_1 G-protein coupled receptors. Researchers from the University of Nottingham, UK, have used BODIPY® 630/650-X succinimidyl ester to synthesize fluorescent analogs of adenosine A_1 receptor (A_1-AR) agonists. The labeled analogs exhibit potent agonist activity demonstrated by radioligand binding and functional analysis in CHO cells expressing human A_1-AR. Ligand-receptor interactions with Topaz fluorescent protein-tagged A_1-AR were visualized by confocal microscopy with simultaneous 633 nm (BODIPY® 630/650 dye) and 488 nm (Topaz FP) excitation.

Middleton, R.J., Briddon, S.J., Cordeaux, Y., Yates, A.S., Dale, C.L., George, M.W., Baker, J.G., Hill, S.J., Kellam, B. (2007) New fluorescent adenosine A_1-receptor agonists that allow quantification of ligand–receptor interactions in microdomains of single living cells. *J Med Chem* 50:782–793.

Correlated light and electron microscopy of apoptotic mitochondria. Ultrastructural transformations of mitochondria specifically associated with cytochrome *c* loss and membrane depolarization in HeLa cells have been identified by researchers from the National Center for Microscopy and Imaging Research at the University of California, San Diego. Sun and coworkers used TC-FlAsH™-tagged cytochrome *c* and the mitochondrial membrane potential sensor tetramethylrhodamine ethyl ester (TMRE) to characterize the state of apoptosis in etoposide-treated HeLa cells, followed by electron microscopy and 3D electron microscope tomography of the identical cells to study the sequence of structural changes. Labeling with TC-FlAsH™ avoids aberrant redistribution of cytochrome *c* (molecular weight ~12 kDa) that may be induced by larger tags such as green fluorescent protein (GFP).

Sun, M.G., Williams, J., Munoz-Pinedo, C., Perkins, G.A., Brown, J.M., Ellisman, M.H., Green, D.R., Frey, T.G. (2007) Correlated three-dimensional light and electron microscopy reveals transformation of mitochondria during apoptosis. *Nat Cell Biol* 9:1057–1065.

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