Neurons in focus
Two-photon excitation imaging in live tissue

Mitochondrial fission and fusion in neurodegeneration

PLUS:
New products for neural stem cell research
BioProbes 60

Features

COVER STORY

4  |  Neurons in focus
Two-photon excitation imaging in live neuronal tissue

NEW TECHNOLOGIES

8  |  RNA as you’ve never seen it before
Detect nascent RNA with Click-IT® RNA Imaging and HCS Assays

10  |  Isolate T cells directly from whole blood
Save time with Dynabeads® FlowComp™ isolation technology

12  |  Chemiluminescent immunodetection and reporter gene assays
High-quality, high-purity substrates for ultrasensitive detection

15  |  Live-cell imaging of cell cycle and cell division
Introducing the Premo® FUCCI Cell Cycle Sensor

16  |  Advances in neural stem cell culture
Superior NSC expansion with StemPro® NSC serum-free medium

18  |  Optimal growth of neurons and neural stem cells
GIBCO® Neurobasal® media and supplements

20  |  Ready-to-use, high-quality neural cells
GIBCO® rat neural primary and stem cells

PRACTICAL APPLICATIONS

22  |  Tools for the study of Alzheimer’s disease
Amyloid beta antibodies, peptides, ELISAs, and Luminex® kits

24  |  A growing list of tools for the violet laser
A guide to violet-excited reagents for flow cytometry

27  |  A fluorogenic, target-specific reagent for small animal in vivo imaging
Alexa Fluor® 680 dye–labeled probe dramatically improves tumor detection, staging, and monitoring

28  |  Interrogating potential drug targets for Parkinson’s disease
Biochemical and cell-based assays for dopamine D2 and LRRK2

30  |  Mitochondrial fission and fusion in neurodegeneration
Approaches for visualizing mitochondrial morphology

32  |  Visualizing tumor metastasis
CellTracker™ dyes, DQ™ collagen, and Geltrex™ matrix

34  |  Innovative solutions for ion channel research
Cell-based and biochemical assays for ion channel activity

38  |  Resources for cytokine research
Antibodies, recombinant proteins, ELISAs, and Luminex® kits

Departments

2  |  JOURNAL HIGHLIGHT
A flow cytometry–based method for isolating neural stem cells

3  |  ON THE WEB
Find your cellular technology at the Cell Resource Center

39  |  JUST RELEASED
Highlighting our newest cellular analysis products and technologies

40  |  ENDNOTE
Recently published: A look at how your fellow researchers are using Invitrogen™ products
A flow cytometry–based method for isolating neural stem cells


What biochemical characteristics distinguish neural stem cells from their nonproliferative neighbors? Despite their relative rarity, neural stem cells serve the vital function of generating new neurons to provide building blocks for neural circuitry—behavior that suggests vast therapeutic potential. In the adult mammalian brain, neural stem cells represent a subset of astrocytes that exhibit few cellular markers to distinguish them from nonproliferative astrocytes, making their isolation and study difficult. However, a subpopulation of stem cell astrocytes found in the subventricular zone (SVZ) has been shown to express the epidermal growth factor receptor (EGFR), providing a strategy for selective purification of activated stem cell astrocytes away from other SVZ neurons.

In this study, Pastrana and colleagues used Alexa Fluor® 647 dye–labeled EGF, combined with FACS analysis, to identify the activated stem cell subpopulation of SVZ astrocytes in mouse brain and successfully purify cells at different stages of the stem cell lineage (Figure 1). This EGFR+ subpopulation was effectively eliminated by pretreatment of mouse brains with the antimitotic drug Ara-C, demonstrating that this subpopulation represents activated stem cell astrocytes. The researchers were also able to combine their analysis with Vybrant® DyeCycle™ dye staining, which allowed the sorting of cells according to their stage in the cell cycle. Further immunostaining and flow cytometric analysis revealed that the isolated subpopulation was as much as 20-fold enriched in cells exhibiting self-renewal and multipotency potential. The authors suggest that this simple purification strategy will greatly facilitate future molecular and functional studies of stem cell astrocytes, in pursuit of a greater understanding of in vivo stem cell behavior and the development of more effective therapeutic strategies.

Learn more about Alexa Fluor® EGF complex and Vybrant® DyeCycle™ Violet stain at www.invitrogen.com/bp60.
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B. Technical resources, protocols, and support tools.
C. The latest news, updates on new products, and promotions.

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Neurons in focus

TWO-PHOTON EXCITATION IMAGING IN LIVE NEURONAL TISSUE.

Nowhere is preserving native cellular context more important than in studies of the nervous system. This intricate 3D structure is at the core of all of the complex functions we observe—disrupt it, and function is altered. Despite the easy access provided by dissociated neurons in culture, especially in optically based investigations such as fluorescence-based calcium imaging [1], the need for more contextual and system-level analysis has driven the development of techniques capable of exploring tissue slices and even the whole brain. Two-photon excitation (TPE) of fluorescent sensors is one approach giving researchers access to deeper layers of functioning cortex (Figure 1). This article reviews data and observations from the literature about the dye choices compatible with TPE, describes what to consider in making those choices, and lists protocols to optimize their use.

Figure 1 (above). Monitoring synaptically evoked calcium transients by two-photon laser-scanning microscopy in intact brain tissue using fluorescent dyes. A CA1 rat pyramidal neuron was microinjected with 300 µM fluo-5F (Cat. No. F14222), a calcium indicator (Kd 800 nM), and 20 µM Alexa Fluor® 594 hydrazide (Cat. No. A10442). Both dyes were excited at 800 nm using two-photon excitation. In the film insets, several frames of calcium transients are shown following individual evoked action potentials in presynaptic axons (evident by the appearance of a yellow-fluorescent signal against the Alexa Fluor® 594 dye–labeled red-fluorescent background). Using a ratio of the two signals, absolute calcium concentration in individual dendritic spines can be calculated. Such high-resolution optical imaging combined with electrophysiology allows hundreds of transmission events to be recorded at identified synapses, providing temporal and spatial precision that would have been unimaginable just a decade ago. Image courtesy of Thomas Oertner, FMI Institute for Biomedical Research, Basel, Switzerland, and Karel Svoboda, HHMI, Janelia Farms Research Institute, Virginia, US.
Advantages of TPE for live-tissue analysis

The practical application of TPE microscopy has advanced considerably through the pioneering work of Watt Webb and his colleagues during the last two decades [2,3]. The principle of TPE was first predicted by Maria Göppert-Mayer in 1931 [4], and it is after her that the TPE unit of absorption cross-section or excitation efficiency is named. It took another three decades and the invention of the pulsed ruby laser, followed by the invention of TPE laser scanning microscopy in 1990 [2], to see TPE emerge as a practical application. The principal advantages are increased depth of resolution (due to the use of near infrared light—700 to 1,100 nm), narrow focal plane of the excitation volume (less than 100 μm), and reduced photobleaching of dye above and below the imaged region (~0.1 μm³) [5]. Thus high-resolution, 3D images of depths up to 1 mm (up to 2 mm in some tissues) can be achieved, compared to less than 200 μm for visible light–based approaches [6] (Figure 2).

The science behind TPE is well reviewed elsewhere [7], but can be conceptualized as a summing of the excitation energies through two long-wave photons (e.g., 1,000 nm) that are focused and concentrated at discrete, small focal volumes (Figure 3). Concentrated in time and space, the two weak photons can sum their energies and excite fluorophores that normally have excitation maxima half that value. Since the excitation beam is concentrated in one focal plane, often as small as 0.1 μm², there is reduced photobleaching above and below the plane of interest, which eliminates phototoxicity. Thus TPE is rapidly becoming a preferred excitation source for physiological analysis of live tissue.

Choosing a fluorophore

In general, any bright one-photon dye can be used in TPE imaging if a somewhat blue-shifted excitation maximum is used (for a complete survey, see the excellent reviews in references 7 and 8). Table 1 lists specific dyes that have been shown to be useful in TPE experiments.

Qdot® nanocrystals have remarkable TPE cross-sectional areas estimated to be three orders of magnitude greater than the best fluorescent dyes or fluorescent proteins. Depending on the size of the Qdot® nanocrystal used, various fluorescent colors can be

Figure 2. Schematic diagram showing a two-photon excitation (TPE) imaging setup and the depths of resolution of commonly used imaging techniques.

Figure 3. An experiment illustrating ordinary (single-photon) excitation of fluorescence and two-photon excitation. The cuvette contains a solution of the dye safranin O, which normally emits yellow light when excited by green light. The upper lens focuses green (543 nm) light from a CW helium–neon laser into the cuvette, producing the expected conical pattern of excitation (fading to the left). The lower lens focuses pulsed infrared (1,046 nm) light from a neodymium–YLF laser. In two-photon absorption, the excitation is proportional to the square of the intensity; thus, the emission is confined to a small point focus (see arrow), which can be positioned anywhere in the cuvette by moving the illuminating beam. Image contributed by Brad Amos, Science Photo Library, London.
FEATURE

Multi-cell bolus labeling (MCBL)

The simplest way to label cells is to use a micropipet to place dye in the region of interest in a technique called multi-cell bolus labeling (MCBL, Figure 2). Easier than achieving either whole-cell patch clamp or intracellular contact, MCBL can be used to deliver calcium-sensing dyes and dyes that selectively label astrocytes (sulforhodamine 101 (SR101) or its fixable version, Texas Red® hydrazide) in one 5-second injection pulse [9,10]. Many cells stained in this way are observed to have taken up the dye within 20 minutes [11].

This approach offers two main benefits: (1) individual cells (micro) as well as groups of cells (macro) can be studied in the same field; and (2) signal can be precisely attributed to astrocytes or neurons. This approach is effective in lower vertebrates and mammals, including whole alert mice and rats, at dye concentrations as low as 100 μM [8]. For this application, the dyes chosen must be cell permeant. Oregon Green® 488 BAPTA-1 AM has been used, but fluo-4 and others can be considered, and, when used with Pluronic® F-127 (Invitrogen Cat. No. P6867) (BASF Corporation), their uptake is increased. It is necessary to consider various outcomes when optimizing the experimental setup: for example, signal gain vs. signal-to-noise ratio, speed of response, the binding affinity of the dye, and color choice. These attributes are summarized in Tables 1 and 2, and a range of options exists. For example, Oregon Green® 488 BAPTA, when equimolar to fluo-4, is three times brighter, so three times less excitation could be used to achieve the same response. Reduced light excitation is a key factor in maintaining cell health. Oregon Green® 488 BAPTA also produces faint staining at low calcium or pre-stimulation levels, which can be helpful in finding the cells. But fluo-4 and its variants are used when a much bigger signal change is needed. For example, 100 μM of fluo-4 or fluo-5F can provide as much as a 50% signal increase in pyramidal cells [7]. For on/off sensing at individual neurons, Oregon Green® 488 BAPTA is a good choice, but for submicron regions like dendritic spines, the greater signal change with fluo-4 is preferred.

Intracellular injection

While more difficult to perform, whole-cell patch clamping and delivery of dyes into cells via microelectrodes allow more precise calcium ion concentration determinations, control of electrical behavior of the

Table 1. Excitable fluorophores for calcium detection for use in two-photon excitation (TPE) microscopy.

<table>
<thead>
<tr>
<th>Fluorophore</th>
<th>$K_d$ in solution at 22–24°C</th>
<th>$\Phi$ TPE cross-section</th>
<th>TPE optimal excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregon Green® 488 BAPTA-1 and -2</td>
<td>170 nM</td>
<td>ND</td>
<td>810–880 nm</td>
<td>530 nm</td>
</tr>
<tr>
<td>Fluo-3</td>
<td>325 nM</td>
<td>10 GM/5 GM</td>
<td>810 nm</td>
<td>526 nm</td>
</tr>
<tr>
<td>Fluo-4</td>
<td>345 nM</td>
<td>ND</td>
<td>800 nm</td>
<td>516 nm</td>
</tr>
<tr>
<td>Fluo-5F</td>
<td>2.3 μM</td>
<td>ND</td>
<td>810 nm</td>
<td>520–530 nm</td>
</tr>
<tr>
<td>Fluo-4FF</td>
<td>9.7 μM</td>
<td>ND</td>
<td>810 nm</td>
<td>516 nm</td>
</tr>
<tr>
<td>Calcium Green™-1 +/- Ca</td>
<td>190 nM</td>
<td>30 GM/2 GM</td>
<td>820 nm</td>
<td>530 nm</td>
</tr>
<tr>
<td>Fura-2 +/- Ca</td>
<td>140 nM</td>
<td>6 GM/0.2 GM</td>
<td>800 nm</td>
<td>505/362 nm</td>
</tr>
<tr>
<td>Indo-1 +/- Ca</td>
<td>230 nM</td>
<td>3.5 GM/1.5 GM</td>
<td>590/700 nm</td>
<td>490/400 nm</td>
</tr>
<tr>
<td>X-rhod-1</td>
<td>0.7 μM</td>
<td>ND</td>
<td>900 nm</td>
<td>602 nm</td>
</tr>
<tr>
<td>X-rhod-5F</td>
<td>1.6 μM</td>
<td>ND</td>
<td>770–800 nm</td>
<td>603 nm</td>
</tr>
<tr>
<td>X-rhod-FF</td>
<td>17 μM</td>
<td>ND</td>
<td>ND</td>
<td>603 nm</td>
</tr>
<tr>
<td>Rhod-FF</td>
<td>19 μM</td>
<td>ND</td>
<td>ND</td>
<td>603 nm</td>
</tr>
</tbody>
</table>

* $K_d$ can change inside cells (see reference 11). Data shown are according to Molecular Probes data taken at 22°C. † Expressed in Göppert-Mayer Units (GM); 1 GM = 10–30 cm–1 s–1. For a more complete listing of TPE experiment–compatible dyes, see www.invitrogen.com/bp60. ND = Not determined.
cell, and delivery of intracellular agonists to second messengers. High affinity dyes such as fluo-4, fluo-5F, X-rhod-1, or X-rhod-5F are used to detect single action potentials or small synapticly evoked signals whereas lower affinity dyes such as fluo-4F, X-rhod-F, or rhod-F are used to sense trains of action potentials or larger synapticly evoked signals [12]. It has been noted that the apparent affinities of nearly all sites in cochlear interneurons [13].

Alternatively, fluorescent protein-tagged protein pairs can be co-injected and FRET interactions studied through lifetime changes, as regulatory elements combine in response to ion fluxes [13]. Tools like these, available from Invitrogen (Tables 1 and 2), are allowing researchers to explore the molecular substrates of learning and memory at the most basic structural level—the minute spines that decorate dendrites throughout the brain. Learn more at www.invitrogen.com/bp60.

### Table 2. Other useful stains for use in two-photon excitation (TPE) microscopy.

<table>
<thead>
<tr>
<th>Stain</th>
<th>Target</th>
<th>° TPE cross-section*</th>
<th>TPE optimal excitation</th>
<th>Emission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexa Fluor® 594 dye</td>
<td>ND</td>
<td>770 nm</td>
<td>516 nm</td>
<td></td>
</tr>
<tr>
<td>Alexa Fluor® Red</td>
<td>NA</td>
<td>ND</td>
<td>570 nm</td>
<td></td>
</tr>
<tr>
<td>Sulforhodamine 101 (SR101) or Texas Red® hydrazide (flexible version of SR101)</td>
<td>Astrocytes</td>
<td>ND</td>
<td>840–890 nm</td>
<td>605 nm</td>
</tr>
<tr>
<td>Alexa Fluor® 594 hydrazide</td>
<td>Ca²⁺-insensitive intracellular marker</td>
<td>ND</td>
<td>810 nm</td>
<td>594 nm</td>
</tr>
<tr>
<td>Fluorescent proteins</td>
<td>General expression tag</td>
<td>100–200 GM</td>
<td>800–1,030 nm</td>
<td>505–610 nm</td>
</tr>
<tr>
<td>Rhodamine B</td>
<td>General cell stain</td>
<td>100–200 GM</td>
<td>840 nm</td>
<td>600 nm</td>
</tr>
<tr>
<td>Lucifer yellow CH L453, L682, L1177</td>
<td>Gap junctions</td>
<td>ND</td>
<td>850 nm</td>
<td>533 nm</td>
</tr>
<tr>
<td>DAPI</td>
<td>DNA/nuclei</td>
<td>0.16 GM</td>
<td>700 nm</td>
<td>450 nm</td>
</tr>
<tr>
<td>FM® 1-43</td>
<td>Vesicle recycling</td>
<td>ND</td>
<td>840 nm</td>
<td>575–600 nm</td>
</tr>
<tr>
<td>Qdot® nanocrystals</td>
<td>NA</td>
<td>Up to 47,000 GM</td>
<td>Broad</td>
<td>8 colors from 545–800 nm</td>
</tr>
</tbody>
</table>

*Expressed in Göppert-Mayer Units (GM): 1 GM = 10⁻⁵⁰ cm⁴ s. † Strongly environment dependent. NA = Not applicable. ND = Not determined.

For a more complete listing of TPE experiment-compatible dyes, see www.invitrogen.com/bp60.

### The future of TPE imaging

Using the TPE technique, experiments considered impossible several years ago are now moving ahead. Possibly the most remarkable and complex of these approaches involves analysis of the contribution of NMDA-induced calcium transients to secondary downstream signaling such as RAS [14] at the level of individual dendritic spines of hippocampal neurons in brain slices. NMDA pulses are delivered with regional accuracy by using the same TPE to uncage MINI-caged glutamate [15]. Alternatively, fluorescent protein–tagged protein pairs can be co-injected and FRET interactions studied through lifetime changes, as regulatory elements combine in response to ion fluxes [13]. Tools like these, available from Invitrogen (Tables 1 and 2), are allowing researchers to explore the molecular substrates of learning and memory at the most basic structural level—the minute spines that decorate dendrites throughout the brain. Learn more at www.invitrogen.com/bp60.

### References

RNA as you’ve never seen it before
DETECT NASCENT RNA WITH CLICK-IT® RNA IMAGING AND HCS ASSAYS.

Click chemistry describes a powerful class of reactions that uses biologically unique moieties to detect biomolecules of interest such as proteins and nucleic acids. This unique technology provides an alternative to traditional immunodetection or radioactive methods. Click reactions possess several characteristics that make them biofriendly: no extreme temperatures or solvents are required, the reaction product is stable, and the components of the reaction are bioinert—the label and the detection tags react only with one another. Although several click reactions are reported in the literature [1,2], copper-catalyzed triazole formation from an azide and an alkyne has emerged as the most widely used click reaction in current biomedical research. Click-it® RNA imaging and Click-it® RNA HCS assays provide everything needed to detect newly synthesized RNA in adherent cells.

Take a snapshot of RNA synthesis with Click-it® RNA assays
Click-it® RNA assays are ideal for imaging global RNA synthesis in multiplex analyses using traditional fluorescence microscopy or high-content screening (HCS). The assays employ an alkyne-modified nucleoside, EU (5-ethylthiouridine), that is fed to cells and incorporated into nascent RNA [3]. The small size of the alkyne tag (MW ~25) enables efficient incorporation by RNA polymerases without any apparent changes to the RNA levels of several housekeeping genes (Figure 1). Detection is accomplished using an Alexa Fluor® azide, which has a small “footprint” (MW <1,000) compared with an IgG antibody (MW ~150,000), and results are available in 30 minutes, eliminating long antibody incubations. Following EU incubation, simply fix and permeabilize, then perform the copper-catalyzed click reaction. Click-it™ RNA assays are compatible with other cell-labeling methods such as immunocytochemistry and small-molecule labeling of cellular structures (Figure 2). The multiplexing

![Figure 1. Housekeeping genes are unaffected in cells incubated with EU. Real-time RT-PCR was conducted to determine if EU incorporation affected housekeeping gene expression levels. RNA was isolated using TRizol® LS reagent (Cat. No. 102906-010) from NIH3T3 cells fed with 0.5 mM EU and 1.0 mM EU. RNA concentrations were determined for each sample, and then 250 ng of RNA and specific primer sets for hACTβ, hHprt1, and hPpib were used in a reaction volume of 50 µL to perform one-step qPCR using the SuperScript® III Platinum® SYBR® Green One-Step qRT-PCR Kit (Cat. No.11736-051) and the Mx3000P system (Stratagene). The threshold cycle (Ct) values were obtained using MxPro software. The data clearly show that mRNA levels of housekeeping genes do not change in the presence of the EU concentrations tested.](image)

![Figure 2. Multiplex imaging with Click-it® RNA assays. NIH3T3 cells were incubated with 1 mM EU for 1 hr, then formaldehyde fixed and permeabilized with Triton® X-100. EU incorporated into newly synthesized RNA (red) in some cells was detected using the Click-it® RNA Alexa Fluor® 594 Imaging Kit (Cat. No. C10330). Tubulin (green) was detected with mouse anti-tubulin IgG (Cat. No. A11126) and visualized with Alexa Fluor® 488 goat anti–mouse IgG (Cat. No. A11001). Nuclei (blue) were stained with Hoechst 33342 (Cat. No. H3570).](image)
Table 1. Quantitative analysis of RNA inhibition.

<table>
<thead>
<tr>
<th>Cell</th>
<th>Alexa Fluor® azide</th>
<th>CV (%)</th>
<th>Signal change</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIH3T3*</td>
<td>Alexa Fluor® 488</td>
<td>6.4</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>Alexa Fluor® 594</td>
<td>10.5</td>
<td>21.4</td>
</tr>
<tr>
<td>HeLa†</td>
<td>Alexa Fluor® 488</td>
<td>8.6</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>Alexa Fluor® 594</td>
<td>19.0</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Cells were treated with 250 nM actinomycin D for 18 hr, and inhibition of RNA synthesis was assayed with the indicated Click-iT® RNA HCS Assay. The data shown represent the average %CV and fold change in signal intensity of treated samples (Max) from either two* or three† Min/Max plates.

capability of the assays makes them ideal for toxicological profiling or interrogation of disease models using high-content imaging platforms (Table 1, Figure 3).

Go viral with Click-it® RNA assays

Little is understood about the sites of viral RNA synthesis, including the transport and fate of nascent RNA inside and outside the viral replication complex [4]. The Click-it® RNA Imaging and Click-it® RNA HCS kits provide exciting new alternatives to antibody-based BrU or BrUTP assays for studying viral RNA synthesis in infected cells (Figure 4). Unlike BrUTP, EU does not require a transfection reagent, and the small size of the Alexa Fluor® azide relative to the anti-BrdU antibody enables detection of both ssRNA and dsRNA. The Click-it® RNA imaging assays provide the tools necessary to determine levels of viral RNA synthesis as well as the subcellular localization of RNA in host cells, which will aid in understanding viral gene expression and genome replication.

Taking a new look at RNA

Click-it® RNA Imaging Kits and Click-it® RNA HCS Assays allow you to apply the advantages of click chemistry to the study of RNA synthesis. Learn more at www.invitrogen.com/bp60.

References


Figure 4. Subcellular localization of RNA in Vero cells. Vero cells were pretreated with 2 µM actinomycin D to inhibit host cell transcription, then infected with Tacaribe virus. Infected cells were incubated with 2 mM EU for 1 hr, followed by cold methanol fixation and permeabilization with Triton® X-100. Nascent RNA (green) was detected with the Click-it® RNA Alexa Fluor® 488 Imaging Kit (Cat. No. C10329). Viral nucleoprotein was detected with a directly labeled Alexa Fluor® 594 monoclonal antibody (red). Colocalization of EU and virus nucleoprotein indicates transcription sites in the host cells (yellow).

Figure 3. Dose response for actinomycin D in HeLa cells using the Click-it® RNA Assay. HeLa cells were treated with the indicated amounts of actinomycin D for 18 hr, followed by a 1 hr incubation with 5-ethynyl uridine (EU). Cells were then fixed and permeabilized, and EU incorporated into newly synthesized RNA was detected using green-fluorescent Alexa Fluor® 488 azide. Quantitative analysis was performed using the Cellomics® ArrayScan® VTI and Compartmental Analysis Bioapplication (Thermo Scientific).

Figure 2. Dose response for actinomycin D using the Click-it® RNA HCS Assay. Cells were treated with the indicated concentrations of actinomycin D for 18 hr, followed by a 1 hr incubation with 5-ethynyl uridine (EU). Cells were then fixed and permeabilized, and EU incorporated into newly synthesized RNA was detected using green-fluorescent Alexa Fluor® 488 azide. Quantitative analysis was performed using the Cellomics® ArrayScan® VTI and Compartmental Analysis Bioapplication (Thermo Scientific).

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Click-it® RNA Alexa Fluor® 488 HCS Assay, 2-plate size</td>
<td>1 kit</td>
<td>C10327</td>
</tr>
<tr>
<td>Click-it® RNA Alexa Fluor® 594 HCS Assay, 2-plate size</td>
<td>1 kit</td>
<td>C10328</td>
</tr>
<tr>
<td>Click-it® RNA Alexa Fluor® 488 Imaging Kit, for 25 coverslips</td>
<td>1 kit</td>
<td>C10329</td>
</tr>
<tr>
<td>Click-it® RNA Alexa Fluor® 594 Imaging Kit, for 25 coverslips</td>
<td>1 kit</td>
<td>C10330</td>
</tr>
<tr>
<td>5-ethynyl uridine (EU)</td>
<td>5 mg</td>
<td>E10345</td>
</tr>
</tbody>
</table>
Isolate T cells directly from whole blood

SAVE TIME WITH DYNABEADS® FLOWCOMP™ ISOLATION TECHNOLOGY.

Fast and easy isolation of pure cells directly from whole blood samples is beneficial for basic research and patient monitoring (e.g., analysis of circulating tumor cells, prenatal diagnosis, T cell enumeration in HIV, chimerism analysis in transplantation) as well as for drug discovery and development. Dynabeads® FlowComp™ technology offers rapid, pure, and gentle isolation of human CD3+, CD4+, and CD8+ T cells directly from whole blood. The beads are released from the cells after isolation to avoid any possible artifacts in your downstream applications.

Dynabeads® FlowComp™ technology

Dynabeads® FlowComp™ technology uses a highly specific biotin-streptavidin technology to positively isolate T cells. Included in the kit is a release buffer that removes the cells from the beads (Figure 1). The process is fast and gentle, ensures very high cell viability, and eliminates the use of columns.

Pure and viable cells isolated directly from whole blood

You can isolate CD3+, CD4+, or CD8+ T cells directly from whole blood, obtaining >95% pure T cells with viability exceeding 98%. There is no need for gradient centrifugation or lysis of red blood cells before isolation, saving several hours compared to isolation from peripheral blood mononuclear cells (PBMCs). Compared with a column-based protocol, the purity and viability of T cells isolated using Dynabeads® FlowComp™ is superior (Figure 2). Plots of forward scatter to side scatter (FSC/SSC) and histograms of the selected population illustrate the difference in purity and the presence of apoptotic and aggregated cells using the column-based method.

Isolated T cells proliferate when activated

To study the proliferative capacity of CD3+ T cells isolated with Dynabeads® FlowComp™ Human CD3, isolated cells were stimulated in vitro using Dynabeads® Human T-Activator CD3/CD28 in a 1:1 bead-to-cell ratio. Proliferation was measured using a Click-IT® EdU assay; from days 2 to 3, more than 49% of the cells had incorporated EdU into newly synthesized DNA (Figure 3), indicating T cell proliferation.

Tetanus toxoid stimulates antigen-specific CD3+ T cell proliferation

CD3+ T cells isolated with Dynabeads® FlowComp™ Human CD3 were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) after isolation and added to PBMCs pulsed with tetanus toxoid. In a proliferation assay, the T cells proliferated in an antigen-specific manner (Figure 4).

“...for the positive isolation of T cells, the kit works beautifully well. I end with a very pure CD3+ population. We demonstrate that the CD3 FlowComp™ kit allows for the isolation of functional T cells and that these cells are capable of being activated in an antigen-specific manner.”

—Amir Horowitz, London School of Hygiene and Tropical Medicine, UK

Dynabeads® FlowComp™ technology—the flexible solution

For more information on mouse and human FlowComp™ products and technology, visit www.invitrogen.com/bp60.
Figure 2. CD3+ T cells isolated with FlowComp™ technology are highly pure and viable. T cells were isolated from whole blood with either Dynabeads® FlowComp™ Human CD3 or a column-based system, then analyzed by flow cytometry. The cells isolated using FlowComp™ technology (P1 area) are a highly uniform population with >99% pure CD3+ T cells (A, B). In contrast, the cells isolated using the column-based method contained more red blood cells (red signal) and smaller cells typical of apoptotic cells or erythrocytes (C). In addition, a CD3+ population with higher FSC is visible, indicating aggregation of isolated T cells (D).

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
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<tbody>
<tr>
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<td>113-61D</td>
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<td>Dynabeads® FlowComp™ Human CD8</td>
<td>Processes 4 x 10^8 cells</td>
<td>113-71D</td>
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<td>Dynabeads® FlowComp™ Human CD3</td>
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<td>113-62D</td>
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<td>Dynabeads® FlowComp™ Flexi</td>
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<td>110-61D</td>
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<td>Dynabeads® Human T-Activator</td>
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<td>111-61D</td>
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<tr>
<td>CD3/CD28 for cell expansion and activation</td>
<td>2 mL</td>
<td>111-31D</td>
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<tr>
<td></td>
<td>5 x 2 mL</td>
<td>111-32D</td>
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<td>DynaMag™-15 magnet</td>
<td>Holds 4 x 5 mL FACS tubes or 4 x 15 mL tubes</td>
<td>123-01D</td>
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<td>DynaMag™-50 magnet</td>
<td>Holds 2 x 50 mL tubes</td>
<td>123-02D</td>
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<td>DynaMag™-2 magnet</td>
<td>Holds 16 x 1.5-2 mL microcentrifuge tubes</td>
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<td>OpTmizer™ T-Cell Expansion SFM</td>
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<tr>
<td>Click-iT™ EdU Alexa Fluor® 647 Flow Cytometry Assay Kit</td>
<td>1 kit</td>
<td>A10202</td>
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Figure 3. Isolated CD3+ T cells proliferate when activated. Freshly isolated CD3+ T cells were stimulated with Dynabeads® Human T-Activator CD3/CD28 for 3 days, and proliferation was measured by the Click-iT™ EdU Alexa Fluor® 647 Flow Cytometry Assay (A); 44% of the cells proliferated, as detected by flow cytometry (B).

Figure 4. Proliferation assay demonstrating tetanus toxoid–specific T cell response. CD3+ T cells isolated by Dynabeads® FlowComp™ Human CD3 were prestained with CFSE, then incubated with antigen-presenting cells (A) with no stimulus or (B) with tetanus toxoid (10 µg/mL). After 120 hr, the cells were analyzed by flow cytometry. (C) shows the nondividing cells from A (green) and the dividing cells from B (red) in a histogram.
Chemiluminescence is the conversion of chemical energy to light energy. 1,2-dioxetanes are one class of synthetic chemical structures that produce light energy upon chemical or enzymatic cleavage. These chemical-based light-producing reactions are employed in a wide variety of laboratory applications, including biological assays, clinical diagnostic assays, biosensors, and hygiene monitoring. These applications are enabled by the high-intensity signal, low background, wide dynamic range, rapid signal production, and assay format compatibility provided by chemiluminescence.

Invitrogen’s high-quality, high-purity 1,2-dioxetane chemiluminescent substrates enable ultrasensitive detection of biomolecules. These substrates are available as stand-alone reagents or in convenient kit format for immunoassays, enzyme assays, reporter gene assays, and cAMP measurement.

Principles of enzyme-activated chemiluminescence

Figure 1 diagrams the production of light by the enzyme-catalyzed decomposition of a 1,2-dioxetane containing a cleavable phosphate residue. In this example, Invitrogen’s CSPD® substrate is dephosphorylated to form a metastable intermediate that produces a phenolate anion that then decomposes, emitting light in the process. The enzyme-catalyzed reaction ultimately produces a steady light glow that can be detected by simple instrumentation or with light-sensitive film. The enhancer partitions water molecules away from the site of signal production, reducing the quenching of the light signal by water molecules and improving overall signal intensity.

Figure 1. The light emission mechanism of CSPD® substrate in the presence of enhancer. The diagram depicts the reaction process for the enzyme-catalyzed decomposition of a 1,2-dioxetane using CSPD® as an example. Following enzymatic cleavage, the resulting product produces a phenolate anion that decomposes, producing light. The enhancer partitions water away from the reactive species to improve light output.

Figure 2. Detection of placental alkaline phosphatase with the NovaBright™ Secreted Placental Alkaline Phosphatase Enzyme Reporter Gene Chemiluminescent Detection Kit. The data demonstrate the linear response of the kit over 5 orders of magnitude.
Advantages of 1,2-dioxetane substrates

Enzyme-triggered 1,2-dioxetane chemiluminescent substrates offer several important advantages. The inherent low background and high signal intensity results in exceptionally high assay sensitivity, allowing femtogram detection (Figure 2). These favorable signal characteristics also result in the exceptional dynamic range of up to 7 orders of magnitude (Figures 2 and 3). Rapid light emission kinetics permits rapid assay completion, often in less than one hour.

Invitrogen's 1,2-dioxetane enzyme substrate-based kits meet the challenging demands of a broad variety of applications. The kits employ CDP-Star® and CSPD® substrates for alkaline phosphatase, and Galacton-Plus® and Galacton-Star® substrates for β-galactosidase. The high quality, purity, and lot-to-lot consistency of these ready-to-use substrates enable excellent reproducibility and low background.

Alkaline phosphatase substrates

CDP-Star® and CSPD® substrates offer key advantages relative to colorimetric and fluorometric methods for detecting alkaline phosphatase or alkaline phosphatase conjugates. For immunoassays, 1,2-dioxetane substrates improve sensitivity and generate results faster than colorimetric methods [1]. These substrates are ideally suited for the detection of placental alkaline phosphatase (PLAP) and secreted placental alkaline phosphatase (SEAP) in reporter gene assays [2,3].

CDP-Star® or CSPD® is incorporated into the Invitrogen™ Chemiluminescent Alkaline Phosphatase ELISA Kits for the construction of direct-capture or competitive immunoassays using an alkaline phosphatase conjugate. The NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit uses the CSPD® substrate to provide highly sensitive detection of the reporter gene product SEAP in a convenient microplate format. The Invitrogen™ cAMP Chemiluminescent Immunoassay Kit also uses the CSPD® substrate in a competitive ELISA assay for the detection of cAMP using either a manual assay or an automated high-throughput screening protocol (Figure 4).

β-Galactosidase substrates

Galacton-Plus® and Galacton-Star® substrates are widely used and have become the gold standards for sensitive quantitation of β-galactosidase in reporter gene assays in both mammalian and yeast cells.
Take advantage of ultrasensitive chemiluminescent detection

Invitrogen’s 1,2-dioxetane enzyme substrate–based kits provide ultrasensitive detection for measuring reporter gene activity, cAMP activity, and alkaline phosphatase ELISAs. Learn more at www.invitrogen.com/bp60.

References


<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Chemiluminescent Alkaline Phosphatase ELISA Kit #1 with CSPD® Substrate and Sapphire™-II Enhancer</td>
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<tr>
<td>Chemiluminescent Alkaline Phosphatase ELISA Kit #2 with CSPD® Substrate and Emerald™-II Enhancer</td>
<td>1,000 assays</td>
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<tr>
<td>Chemiluminescent Alkaline Phosphatase ELISA Kit #3 with CDP-Star® Substrate and Sapphire™-II Enhancer</td>
<td>1,000 assays</td>
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<tr>
<td>Chemiluminescent Alkaline Phosphatase ELISA Kit #4 with CDP-Star® Substrate and Emerald™-II Enhancer</td>
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<td>Chemiluminescent Alkaline Phosphatase ELISA Substrate/Enhancer Sampler Kit</td>
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<td>NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit</td>
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<td>NovaBright™ Secreted Placental Alkaline Phosphatase (SEAP) Enzyme Reporter Gene Chemiluminescent Detection Kit</td>
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<td>NovaBright™ β-Galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit</td>
<td>200 assays</td>
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<td>NovaBright™ β-Galactosidase and Firefly Luciferase Dual Enzyme Reporter Gene Chemiluminescent Detection Kit</td>
<td>600 assays</td>
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<tr>
<td>NovaBright™ β-Galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit for Mammalian Cells</td>
<td>200 assays</td>
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<tr>
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<td>NovaBright™ β-Galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit for Yeast Cells</td>
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<td>NovaBright™ β-Galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit for Yeast Cells</td>
<td>1,000 assays</td>
<td>N10566</td>
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</table>

The chemiluminescent substrates for β-galactosidase exhibit over 3 orders of magnitude greater sensitivity than colorimetric assays.

NovaBright™ β-Galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kits for Mammalian or Yeast Cells use Galacton-Plus® in combination with the luciferase substrate, luciferin, in a dual reporter enzyme detection assay. A single cell lysate sample is used, and the light emission for each enzyme is measured sequentially (Figure 3). The kit allows the detection of reporter gene activity from the same sample with a large dynamic range (6–7 orders of magnitude).

Figure 5. Sensitivity of the NovaBright™ β-Galactosidase Enzyme Reporter Gene Chemiluminescent Detection Kit. β-galactosidase assays using purified β-galactosidase diluted in PBS showing linearity over 4 orders of magnitude of enzyme. Assays were measured on a microplate luminometer.
Live-cell imaging of cell cycle and cell division

INTRODUCING THE PREMO™ FUCCI CELL CYCLE SENSOR.

The Premo™ FUCCI Cell Cycle Sensor enables live-cell imaging of cell cycle and division—as cells progress through the cell cycle, nuclear fluorescence changes from red to green. Based on the BacMam gene delivery system, the genetically encoded and prepackaged fluorescent protein (FP)–based reagents are ready for immediate use and optimization for your live-cell applications.

Color-coded G0/S and G2/M

Miyawaki and colleagues developed the fluorescence ubiquitination cell cycle indicator (FUCCI), a sensor that uses two different FPs fused to regulators of the cell cycle: Cdt1 and geminin [1]. Ubiquitin E3 ligases add ubiquitin to Cdt1 and geminin, thereby targeting these proteins to the proteasome for degradation. Temporal regulation of E3 ligase activity results in the biphasic cycling of geminin and Cdt1 through the cell cycle. In G0, geminin is degraded, leaving only Cdt1 tagged with Red Fluorescent Protein (RFP), resulting in red-fluorescent nuclei. During the G0/S transition, Cdt1 levels fall as geminin levels rise; because both proteins are present, both Green Fluorescent Protein (GFP) and RFP fluorescence is visible, and the cell appears yellow when green and red images are overlaid. In S and G2 into M phase, Cdt1 is degraded, leaving only geminin tagged with GFP, so that cells in G2 and M phase appear green. This color change from red to yellow to green serves as an important marker of progression through cell cycle and division (Figure 1A).

Figure 1. Imaging cell cycle progression in live cells with Premo™ FUCCI Cell Cycle Sensor. (A) Schematic of cell cycle progression with nuclear fluorescence changes. (B) U2OS cells were transduced with Premo™ FUCCI Cell Cycle Sensor. Images were collected over 15 hours.

Ready-to-use Premo™ FUCCI Cell Cycle Sensor

Invitrogen has combined FUCCI with the powerful BacMam gene delivery system to form the Premo™ FUCCI Cell Cycle Sensor. BacMam uses a modified baculovirus that contains the FP chimeras. The genetically encoded and prepackaged reagents are ready for immediate use—there’s no need to purify plasmid or worry about vector integrity and quality. Transduction is efficient and reproducible in most cell types, including primary and stem cells, without apparent cytopathic effects. No lipids, dye-loading chemicals, or other potentially harmful treatments are required. In addition, BacMam technology allows you to precisely titrate expression levels to optimize the reagent for your experiment. Simply add the reagent to your cells for 1–2 hours, treat with the enhancer for 1–2 hours, wash, incubate overnight, then visualize cell cycle progression in populations of cells using fluorescence microscopy or high-throughput imaging platforms (Figure 1B). Learn more about the Premo™ FUCCI Cell Cycle Sensor at www.invitrogen.com/bp60.

Reference


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<tr>
<th>Product</th>
<th>Quantity</th>
<th>Cat. No.</th>
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<tr>
<td>Premo™ FUCCI Cell Cycle Sensor</td>
<td>1 kit</td>
<td>P36232</td>
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Advances in neural stem cell culture

SUPERIOR NSC EXPANSION WITH STEMPRO® NSC SERUM-FREE MEDIUM.

Neural stem cells (NSCs) are self-renewing, multipotent cells of the nervous system that can differentiate into neurons or glial cells (oligodendrocytes and astrocytes). NSCs can be isolated from the fetal or adult central nervous system (CNS) \[1,2\] or derived from embryonic stem cells (ESCs) \[3,4\]. Isolated adult NSCs are often limited in their differentiation potential, with the propensity to differentiate toward the predominant neural lineage surrounding the NSCs prior to isolation. In contrast, ESC-derived NSCs have the capacity to differentiate into all three lineages. Because of their versatility, NSCs are a valuable tool for basic research and for clinical applications to treat neurodegenerative diseases and neurological disorders.

When NSCs were first isolated from adult tissue, scientists initially relied on serum-containing media for NSC culture and expansion \[5\]. NSCs have the propensity to differentiate into neurons and glial cells in serum-containing media, and the development of serum-free alternatives such as the GIBCO® Neurobasal® Medium/N-2 combination alleviated this problem. However, the stringent culture conditions required with this system often resulted in suboptimal expansion of NSCs.

To address the need for a serum-free solution optimized for both fetal- and ESC-derived NSCs, we developed StemPro® NSC SFM, enabling superior NSC expansion efficiency of adherent and neurosphere suspension cultures while maintaining normal NSC multipotency, phenotype, and karyotype.

Proliferation of human neural stem cells in StemPro® NSC SFM

Human NSCs (hNSCs) represent a very small fraction of the total CNS cell population, thus, expansion is critical for generating a sufficient number of cells to study differentiation pathways and explore the downstream clinical applications hNSCs. In addition, hNSCs can be passaged only a limited number of times before exhibiting reduced proliferation and differentiation potential; therefore, maximizing the total hNSC yield per passage is essential.

To evaluate StemPro® NSC SFM performance, we compared the proliferation rates of hESC-derived NSCs grown in StemPro® NSC SFM vs. Neurobasal® Medium and competitor media, and analyzed the expression of key NSC markers. NSCs cultured in StemPro® NSC SFM proliferated at higher rates than NSCs grown in Neurobasal Medium or competitor media (Figure 1). NSCs grown in StemPro® NSC SFM expressed the NSC markers Nestin and Sox2 (Figure 2), demonstrating

![Figure 1. Proliferation of hNSCs cultured in StemPro® NSC SFM, competitor medium 1, competitor medium 2, or Neurobasal™ N2-supplemented medium. ESC-derived hNSCs were seeded at 1 x 10^4 cells per well in CELLstart™ substrate–coated 96-well plates for 3 days in each respective medium. Indirect cell counts were obtained with the CyQUANT® Cell Proliferation Assay Kit (Cat. No. C35006). Data represent the mean relative florescence units from stained cells (n = 6).](image1)

![Figure 2. Phenotype marker expression in neural stem cells cultured in StemPro® NSC SFM through passage 17. NSCs expressed the normal phenotypic markers Nestin (A) and Sox2 (B), in the absence of ESC marker Oct4. The inset image in each panel shows DAPI nuclear staining.](image2)
retention of the NSC phenotype. Maintenance of NSC karyotype was also shown (Figure 3).

Differentiation potential of NSCs cultured in StemPro® NSC SFM

hNSCs are defined by the ability to differentiate to three distinct lineages—neurons, oligodendrocytes, and astrocytes. To demonstrate that hNSCs cultured in StemPro® NSC SFM retain their multipotent differentiation potential, we induced differentiation of hNSCs cultured in StemPro® NSC SFM to these three lineages (Figure 4). We also examined whether the neurons were physiologically active using an indirect measurement of action potential. Neurons emitted fluorescence upon treatment with the neurotransmitters acetylcholine, glutamate, norepinephrine, and ATP, demonstrating activity (Figure 5).

Learn more about Invitrogen’s neural stem cell products

StemPro® NSC SFM enables superior expansion of human and rodent NSCs derived from either embryonic stem cells or fetal tissue, and supports long-term growth and expansion of both adherent and neurosphere suspension cultures. StemPro® NSC SFM is produced under cGMP regulations and is qualified using an hNSC performance assay, providing excellent batch-to-batch consistency. For more information about StemPro® NSC SFM and other products for neurobiology, visit www.invitrogen.com/bp60.

References


<table>
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<th>Product</th>
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<td>StemPro® NSC SFM</td>
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<tr>
<td>CELLstart™ substrate</td>
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<td>Neurobasal™ Medium (1X)</td>
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<td>21103049</td>
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<tr>
<td>B-27 Serum-Free Supplement (50X)</td>
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During the last two decades, the field of neurobiology has seen great advances, leading to effective treatments for diseases such as Alzheimer’s, Parkinson’s, and multiple sclerosis. Research in these areas has been enabled by the availability of Gibco® serum-free neural cell culture products such as Neurobasal® Medium, B-27 Supplement, N-2 Supplement, G-5 Supplement, and the new StemPro® Neural Supplement. These cGMP-manufactured products support optimal growth of neural stem cells and all the major neural cell types (Table 1).

### Neurobasal® media

Neurobasal® media are serum-free formulations that meet the special requirements of neuronal cells [1–4]. When supplemented, they support the long-term growth and normal phenotypes of neuronal cells, and enable highly enriched populations of neuronal cells to be maintained in the absence of an astrocyte feeder layer. Neurobasal® media are available in two formulations: Neurobasal® Medium is optimized for long-term growth of fetal hippocampal neurons and many other neurons of the CNS and PNS; Neurobasal®-A Medium is optimized for the long-term growth and viability of postnatal and adult neurons of the CNS and PNS. For added stability, Neurobasal® media are formulated without L-glutamine. Addition of a serum-free supplement, discussed in the following sections, is required before use.

#### B-27 Supplement

B-27 Supplement [5–12] is an optimized serum substitute that supports the long-term growth and viability of hippocampal neurons in Neurobasal® Medium without an astrocyte feeder layer (Figure 1). The lack of the excitatory amino acids glutamate and aspartate makes this supplement ideal for neurotransmitter response studies. Because of these

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**Table 1. Choosing the right medium for your neural cell type.**

<table>
<thead>
<tr>
<th>Neuronal cell type</th>
<th>Basal media</th>
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<tr>
<td>Neurons</td>
<td>Neurobasal®, Neurobasal®-A, DMEM, DMEM/F-12</td>
<td>B-27, N-2, StemPro®</td>
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<td>Glial cells</td>
<td>Oligodendrocytes, radial and other specialized glia</td>
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<tr>
<td><strong>PNS</strong></td>
<td></td>
<td></td>
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<tr>
<td>Neural crest</td>
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<tr>
<td>Placodes</td>
<td>Cranial ganglion (ears and eyes)</td>
<td>Neuronal*, DMEM/F-12</td>
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<tr>
<td><strong>Other</strong></td>
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<td>Stem cells</td>
<td>Neuronal stem cells, neural precursor cells, neuroepithelial cells</td>
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<td>Cell lines</td>
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<td>Microglial cells</td>
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<td>B-27, G-5</td>
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</table>

* G-5 Supplement can also be used to support the growth of astrocytes.

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Figure 1. Immunofluorescence detection of primary rat cortex neurons (A) and primary rat hippocampus neurons (B). Primary neurons were cultured in Neurobasal® Medium supplemented with B-27 and GlutaMAX™-I for 1 week. Neurons were labeled with mouse anti-MAP2 antibody and detected using Alexa Fluor® 488 goat anti–mouse IgG antibody (green). Nuclei were stained with DAPI (blue).
properties, researchers have extended applications of B-27 to the culture of neurons from various brain parts including striatum, substantia nigra, septum, cerebral cortex, cerebellum, and dentate gyrus, as well as neural stem cells, neuronal tumor cell lines, and some nonneuronal cell types.

B-27 without antioxidants, or without retinoic acid
B-27 Supplement Minus AO excludes antioxidants that could interfere with the analysis of free radical damage, apoptosis, or age-related neurodegenerative diseases. B-27 Supplement without retinoic acid is available for the growth of CNS progenitors or for stem cells that require controlled levels of vitamin A in the culture.

StemPro® Neural Supplement
Due to their scarce availability, the in vitro expansion of neural stem cells (NSCs) and neural progenitor cells has been a challenge to academic and clinical researchers. To address this challenge, we developed StemPro® Neural Supplement—a convenient and cost-effective serum-free supplement for optimal proliferation of human and rat NSCs and neural progenitor cells such as glial precursor cells (GPCs) (Figure 2).

N-2 Supplement
N-2 Supplement is a chemically defined additive for Neurobasal® media. N-2 can be used for the growth of rat primary embryonic neurons, embryonic neurospheres, and CNS progenitor cells when combined with the growth factors bFGF and EGF in Neurobasal® Medium or DMEM. N-2 can also be used for the growth and expansion of post-mitotic neurons and tumor cells with neuronal phenotypes [13–15].

G-5 Supplement
G-5 Supplement is a chemically defined additive for the growth of glial cells in Neurobasal® media or other basal formulations such as DMEM, DMEM/F-12, and EMEM. G-5 also supports growth of the primary and senil tumor lines of the astrocytic phenotype [16,17].

Products for optimal neural cell culture
Learn more about Neurobasal® media, supplements, and other related products for neural cell culture at www.invitrogen.com/bp60.
New Technologies

Ready-to-use, high-quality neural cells
GIBCO® Rat Neural Primary and Stem Cells.

As an alternative to freshly isolated neural primary cells and neural stem cells (NSCs), Invitrogen now offers GIBCO® rat fetal neural stem cells, rat glial precursor cells, rat primary cortical astrocytes, and rat primary cortex and hippocampal neurons. These ready-to-use cells have been developed for optimal performance with high purity, minimal lot-to-lot variability, and superior post-thaw viability.

Rat fetal neural stem cells
GIBCO® rat fetal neural stem cells are isolated from the cortex of Sprague-Dawley rats at day 14 of gestation, and can be used for both adherent cell culture and neurosphere suspension culture. These cells can be expanded in StemPro® NSC SFM (see page 16) for up to 3 passages without differentiation. During expansion, more than 75% of the NSCs retain their undifferentiated phenotype (Figure 1). GIBCO® Rat Fetal NSCs can also spontaneously differentiate into neurons, oligodendrocytes, and astrocytes as shown by immunocytochemistry.

Rat glial precursor cells
Glial precursor cells (GPCs) are restricted progenitors; the majority of their downstream progeny are oligodendrocytes and astrocytes. GIBCO® rat glial precursor cells are isolated from the cortex of newborn Sprague-Dawley rats. More than 80% of the GPCs retain the undifferentiated phenotype marker A2B5 upon proliferation (Figure 2), and retain the ability to differentiate into oligodendrocytes and astrocytes.

Rat primary cortical astrocytes
Astrocytes constitute the most numerous cell type in the central nervous system (CNS), and have critical roles in adult CNS homeostasis. GIBCO® rat primary cortical astrocytes are isolated from the cortex of

Figure 1. Marker expression in undifferentiated neural stem cells (NSCs). Rat fetal NSCs were cultured in StemPro® NSC SFM for 10 days to passage 3. While approximately 90% of the cells stained positive (red) for the undifferentiated NSC marker nestin (A), less than 10% of the cells stained positive for differentiated cell type markers doublecortin (DCX) (<5%) (B), GalC (<5%) (C), and GFAP (<8%) (D). Nuclei were stained with DAPI (blue) in all images. Magnification: 20x.

Figure 2. Rat glial precursor cells (GPCs) stained by indirect immunofluorescence for the cell surface marker A2B5 (green). Nuclei were stained with DAPI (blue). Cells were maintained in the undifferentiated state in GPC recovery media for 3 days prior to 4% paraformaldehyde fixation and staining.
Figure 3. GFAP expression in rat cortical astrocytes. Cells were cultured for 3 days to passage 2. While approximately 80% of the cells stained positive (red) for the astrocyte marker GFAP (A), less than 10% of the cells stained positive for the immature neuronal marker doublecortin (DCX) (B) and the oligodendrocyte marker galactosylceramide (GaC) (C). Nuclei were stained with DAPI (blue) in all images. Magnification: 20x.

Sprague-Dawley rats at day 19 of gestation and are cryopreserved at the end of first passage. These astrocytes demonstrate superior post-thaw viability and can be further expanded for at least two additional passages. More than 80% of GIBCO® rat primary cortical astrocytes stain positive for the astrocyte-specific marker glial fibrillary acidic protein (GFAP) upon expansion (Figure 3).

Rat primary cortex and hippocampus neurons
GIBCO® rat primary cortex and hippocampus neurons are isolated from Fischer 344 rats at gestation day 18, then cryopreserved in a medium containing 10% DMSO. These cells exhibit high purity with a low glial cell background (<10%). Neurons are extremely sensitive to the freezing and thawing process, which can result in cell death. However, GIBCO® rat primary cortex and hippocampus neurons consistently exhibit post-thaw viability in the range of 50–80%, unlike competitor neurons, which exhibit post-thaw viability in the range of 4–20% (Figure 4).

Find the right neural stem cells for your research
Learn more about primary and stem cell systems available for your research at www.invitrogen.com/bp60.

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
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<td>GIBCO® Rat Fetal Neural Stem Cell Kit</td>
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<td>GIBCO® Rat Glial Precursor Cells</td>
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<td>GIBCO® Rat Primary Cortical Astrocytes</td>
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<td>GIBCO® Rat Primary Cortex Neurons (~4 x 10^6 cells)</td>
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<td>GIBCO® Rat Primary Hippocampus Neurons</td>
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Tools for the study of Alzheimer’s disease

AMYLOID BETA ANTIBODIES, PEPTIDES, ELISAS, AND LUMINEX® KITS.

Alzheimer’s disease is a neurodegenerative disorder characterized by the aggregation of the amyloid beta (also known as Aβ or β-amyloid) peptide in the brain. This peptide, a 39 to 43 amino acid cleavage product of the amyloid precursor protein (APP), is the major constituent of the protein plaques observed in the brains of Alzheimer’s patients. Because the disease is currently incurable, many efforts are focused on understanding the role of amyloid beta in the onset and progression of the disease. Aβ antibodies, synthetic peptides, ELISAs, and Luminex® kits are valuable tools for the detection and characterization of APP and Aβ cleavage isoforms. Here we provide an overview of these tools and describe their applications for Alzheimer’s disease research.

Amyloid beta antibodies

Cleavage of APP by β-secretase generates a variety of Aβ isoforms, ranging from 39 to 43 amino acids in length (Figure 1). The most common isoforms are Aβ40 (Aβ[1–40]) and Aβ42 (Aβ[1–42]), with Aβ42 having a greater tendency to aggregate. A wide selection of antibodies targeting both APP and its cleavage isoforms is available (Table 1). These antibodies are available both unconjugated and biotin-labeled, from a variety of sources.

Table 1. Anti-APP and anti-At antibodies.

<table>
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<th>Antibody</th>
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<th>Applications</th>
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*Reactivity predicted but not tested

Reactive species: Hu = human, Ms = mouse, Rt = rat
Applications: DB = dot blot, E = ELISA, IC = immunocytochemistry, IF = immunofluorescence, IHC = immunohistochemistry, IP = immunoprecipitation, RIA = radioimmunoassay, WB = western blot.
host species, and are validated for diverse applications including western blotting, immunoassays, immunoprecipitation, immunocytochemistry, and immunohistochemistry. Immunostaining of Aβ enables the detection of Aβ deposits in the brains of human Alzheimer’s disease patients or in animal model systems (Figure 2). Anti-Aβ monoclonal antibodies have also been shown to reduce amyloid plaque formation in vitro and in animal studies [1,2].

**Amyloid beta peptides**

Because amyloid plaques are usually too fragile to extract from the brains of diseased patients, researchers have developed techniques to study synthetic plaque assembly and growth in vitro [3]. Invitrogen offers Aβ40 and Aβ42 synthetic peptides in addition to an inactive control peptide for in vitro studies of Aβ aggregation and deposition (Table 2).

**Table 2. Aβ synthetic peptides and peptide fragments.**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Quantity</th>
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<td>Aβ [1–40], ultra pure</td>
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<td>03243</td>
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<tr>
<td>Aβ [40–1] (inactive control) peptide</td>
<td>1 mg</td>
<td>03245</td>
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<tr>
<td>Aβ [40–1] (inactive control) peptide, biotin</td>
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<td>Aβ [1–40, Gly5, Phe10, Arg13] peptide, rat homolog</td>
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<td>Aβ [1–42] peptide</td>
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<td>Aβ [1–42] peptide</td>
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<tr>
<td>Aβ [42–1] (inactive control) peptide</td>
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<td>03247</td>
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<td>Aβ [1–28] peptide fragment</td>
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**Amyloid beta ELISAs and Luminex® kits**

Enzyme-linked immunosorbent assays (ELISAs) [4] or Luminex® bead-based assays [5] can be used for sensitive, quantitative detection of APP, Aβ, and α-synuclein, another component of Alzheimer’s plaques, from cerebrospinal fluid (CSF), brain homogenate, or tissue culture supernatant samples (Table 3). In a recent study, the levels of Aβ42 and the tau protein in CSF were found to correlate with the transition from latent Alzheimer’s disease to the onset of minor cognitive impairment [5].

**Advancing neuroscience research**

Start exploring Invitrogen’s comprehensive selection of tools for neuroscience research, including antibodies, fluorescent probes, neural primary and stem cells, and neural media. Learn more at www.invitrogen.com/bp60.

**References**

Violet lasers are critical to achieving the full analytical capability of modern flow cytometry. Violet-excited reagents allow researchers to take significant steps forward in the analysis of cellular function—far beyond what has been achieved with immunophenotyping alone. Here we describe a comprehensive collection of Molecular Probes® violet-excitable reagents and assays for immunophenotyping, viability, cell cycle, and apoptosis. Because these reagents are excited by your flow cytometer’s violet laser, you have more options when using the crowded blue and red excitation lines (Table 1).

**Multicolor immunophenotyping**

Immunophenotyping applications represent the majority of flow cytometer use. Violet-excitable reagents allow three to four colors to be easily added to standard antibody panels. Pacific Blue™ dye (emission maximum ~455 nm) is commonly used and can easily be paired with Pacific Orange™ dye (emission maximum ~551 nm) (Figure 1A). Both dyes are available as direct, Zenon®, and second-step conjugates.

**Viability and vitality dyes**

Nonspecific staining from damaged cells can interfere with data collected on critical cell populations; the use of viability dyes allows you to exclude dead cells from analysis. SYTOX® Blue Dead Cell Stain (Cat. No. S34857), a traditional viability reagent, is a high-affinity dye that exhibits >500-fold fluorescence enhancement upon binding to nucleic acid. The dye easily penetrates cells with compromised plasma membranes but will not cross intact cell membranes.

Fixable dead-cell dyes comprise a new class of viability reagents that covalently react with available amines. In healthy cells, these dyes are excluded from the interior and therefore only label surface proteins. In dead cells they enter and label proteins throughout the cytoplasmic volume. Dead cells have at least 50-fold greater fluorescence than live cells, and the covalently labeled cells can be aldehyde-fixed and permeabilized for intracellular staining protocols without losing viability discrimination (Figure 2). There are three options for use with violet excitation: LIVE/DEAD® Fixable Violet (~460 nm emission, Cat. No. L34955), Aqua (~530 nm emission, Cat. No. L34957), and Yellow (~560 nm emission, Cat. No. L34959) Dead Cell Stains. In addition, Arc™ amine-reactive beads (Cat. No. A10346) are now available to help researchers set compensation with fixable dead-cell reagents.

Cell vitality can be measured by the esterase activity that is ubiquitous in the cytosol of live cells. Cell-permeant calcein violet AM (Cat. No. C34858) is cleaved by esterases to form highly fluorescent calcein violet (emission ~452 nm), which is retained in the cell.

---

**Figure 1. Violet-excited fluorophores for antibody staining.** Human peripheral blood leukocytes (PBLs) were stained with either (A) Pacific Blue™ anti-CD4 and Pacific Orange™ anti-CD8 or (B) Qdot® 605 anti-CD4 and Qdot® 655 anti-CD3 antibodies. Samples were analyzed on a BD LSR™ II flow cytometer using violet diode laser excitation. Plots are gated on lymphocytes by scatter. Axes are labeled with the bandpass filters used; plots are labeled with compensation values (arrows).
Cell cycle analysis

There are several dyes available for analyzing the DNA distribution in both fixed and viable cells. When loaded, these dyes allow the clear distinction of the different phases of the cell cycle: G0/G1 (2N), S (DNA replication), and G2/M (4N). FxCycle™ Violet Stain (Cat. No. F10347) is a violet laser–excited dye used for cell cycle analysis in fixed cells (Figure 3) that exhibits little spectral overlap into channels of other lasers.

Vybrant® DyeCycle™ Violet (emission maximum ~440 nm, Cat. No. V35003) is used for DNA content analysis in live cells. DyeCycle™ Violet Stain is DNA selective, cell membrane–permeant, and essentially non-fluorescent until bound to double-stranded DNA. Vybrant® DyeCycle™ Violet Stain is excited by 405 nm and near-UV laser lines. This live-cell dye is essentially nontoxic and offers the possibility of cell sorting based on DNA content. Vybrant® DyeCycle™ Violet Stain has also been shown to identify stem cell side populations in hematopoietic tissues using the violet laser [1] and can be used to detect condensed chromatin in studies of apoptosis (see Table 1).

Cell proliferation assays

Nucleoside incorporation provides direct measurement of new DNA synthesis in cells and has traditionally been detected by
incorporation of the nucleoside analog bromodeoxyuridine (BrdU) into DNA, followed by the addition of an anti-BrdU antibody. 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. The Click-iT® EdU Pacific Blue™ Flow Cytometry Assay Kit (Cat. No. A10034) eliminates the need to denature DNA. The Click-iT® advantage is in the chemistry—small, unique, and low-background labeling and detection moieties that react specifically and covalently with one another. 5-ethyl-2’-deoxyuridine (EdU) is a nucleoside analog containing an alkyne. In a copper-catalyzed reaction, the alkyne reacts with a Pacific Blue™ dye–labeled azide, forming a stable covalent bond. The small size of the azide reagent allows efficient access to the DNA without the need for harsh cell treatment, which simplifies the assay considerably but delivers the same results (Figure 4). Click-iT® EdU labeling is compatible with most fixation protocols.

Detection of apoptosis

Apoptosis is the carefully regulated process of cell death that occurs as a part of normal development. Three of our apoptosis kits incorporate violet-excitable stains. The Pacific Blue™–annexin V conjugate (Cat. No. A35136) detects changes in membrane asymmetry characteristic of apoptotic cells (Figure 5A). Slight changes in permeability to dyes such as PO-PRO™-1 dye (Cat. No. A35123) can also be used to identify apoptotic cells (Figure 5B). Unlike annexin conjugates, PO-PRO™-1 dye can provide efficient staining of both trypsinized adherent and suspension cells [2]. In addition, DyeCycle™ Violet stain (Cat. No. A35135) can detect chromatin condensation, a late event in apoptosis (Figure 5C).

A growing list of violet-excited reagents

The list of Molecular Probes® violet laser–compatible applications continues to grow as we develop new fluorophores specifically for this laser. In addition to the applications above, violet excitation can be used with Fura Red™ dye for measurement of calcium flux and with CountBright™ Absolute Counting Beads to quantify cell concentrations in samples. As a result, researchers will be able to easily expand their multispectral capabilities on flow cytometers. Find out more about our entire line of violet-excitable reagents at www.invitrogen.com/bp60.

References


Figure 4. Flow cytometric analysis of cell proliferation using Click-iT® technology. Jurkat cells were treated with 10 μM EdU for 2 hr, then fixed and permeabilized, stained via the click reaction, washed, and counterstained for cell cycle analysis using 7-amino-actinomycin D (7-AAD). Cells were analyzed using a flow cytometer with 405 and 488 nm excitation.

Figure 5. Violet-excited detection of apoptosis. Jurkat cells were treated with 10 μM camptothecin for 4 hr and then stained with (A) the reagents in the Apoptosis Kit–Pacific Blue™ Annexin V/SYTOX® AADvanced™, (B) PO-PRO™-1 and 7-AAD stains, which can be used for adherent or suspension cells, or (C) Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ stains. Cells were analyzed by flow cytometry using 405 nm and 488 nm excitation. Viable (V), apoptotic (A), and necrotic (N) populations are labeled in each plot.
A fluorogenic, target-specific reagent for small animal in vivo imaging

ALEXA FLUOR® 680 DYE–LABELED PROBE DRAMATICALLY IMPROVES TUMOR DETECTION, STAGING, AND MONITORING.

Fluorescent near-infrared (NIR) dye–based in vivo imaging holds significant promise for understanding tumor growth and treatment strategies. However, creating fluorescent bioconjugates suitable for in vivo studies is difficult due to intramolecular quenching and rapid clearance through the liver. To avoid these issues, we offer SAIVI™ antibody/protein kits that control the degree of labeling (DOL) to 2–3 dye molecules per protein. But in a recent paper, researchers approach the problem in reverse—rather than avoiding intramolecular quenching, the team intentionally causes quenching by overlabeling an antibody with Invitrogen’s Alexa Fluor® 680 dye. The result is a highly specific, fluorogenic reagent that targets only tumor cells and is not cleared to the liver.

Creating a targeted, fluorogenic NIR reagent for tumor imaging

Ogawa and coworkers detail a protocol using Alexa Fluor® 680 dye and a therapeutic, humanized monoclonal HER-2 antibody, trastuzumab (Genentech) [1]. By overlabeling with 7–9 fluorophores per protein molecule, the authors create a quenched probe that is not cleared to the liver. This probe, when injected into animals or presented in vitro, targets only the tumor cells; after binding to the cells, the probe is internalized. When the complex degrades, only the affected cells fluoresce as the quenching interactions are uncoupled. Traditional nonquenched probes contribute generalized fluorescence at the tumor site due to leaky vasculature, a hallmark of tumor neo-vessels. By creating a quenched probe, the authors overcame this barrier; the probe was only detectable when bound and internalized by targeted cells (Figure 1).

When the authors compared Alexa Fluor® 680 dye–labeled conjugates to those prepared using an equivalent NIR dye, Cy®5.5 (GE Healthcare), they found that only the Alexa Fluor® dye–based probe showed high levels of specific targeting and remained in circulation, avoiding rapid clearance through the liver (data not shown).

Two conjugation strategies for in vivo imaging with NIR dyes

Our scientists have previously demonstrated that a high DOL causes rapid clearance to the liver when mouse and rabbit antibodies are used for in vivo imaging. However, humanized therapeutic antibodies have been designed to stay in circulation for days, and this paper shows that overlabeling these antibodies with Alexa Fluor® 680 dye—although not with other NIR dyes—does not increase clearance to the liver.

Thus, two conjugation strategies are available for NIR dye–based in vivo imaging. The SAIVI™ Rapid Antibody Labeling Kits may be best suited for research applications using mouse and rabbit antibodies, where internalization and degradation are not expected, to ensure low DOL, minimal clearance to the liver, and minimal quenching. Using a high DOL with the Alexa Fluor® 680 dye, as described by Ogawa and coworkers, should be considered for humanized monoclonal antibodies that are intended to be internalized, and thus unquenched. Learn more about Alexa Fluor® dyes for in vivo imaging at www.invitrogen.com/bp60.

Reference

Interrogating potential drug targets for Parkinson’s disease

**BIOCHEMICAL AND CELL-BASED ASSAYS FOR DOPAMINE D2 AND LRRK2.**

Parkinson’s disease (PD), a degenerative disorder of the central nervous system caused by a loss of dopamine-secreting neurons, results in altered neuronal activity within regions of the brain that regulate movement. Classical symptoms of the disease include resting tremors, bradykinesia, rigidity, and postural instability. Current therapies have focused on treating the symptoms of PD, however, recently identified genetic mutations may spur the development of new therapies to address the cause of the disease. This complex disease involves many cellular components and pathways, offering a variety of potential targets—including the G-protein–coupled receptor (GPCR) dopamine D2 and the serine/threonine protein kinase LRRK2 (leucine-rich repeat kinase 2)—for drug therapeutics (Figure 1). Invitrogen offers biochemical and cell-based assays for studying these key targets within the pathways regulating PD. Alternatively, you can outsource your screening and profiling projects to Invitrogen’s SelectScreen® Service.

**Measuring dopamine D2 activation using GeneBLAzer® technology**

The first-line treatment for PD is the use of dopamine agonists, many of which act through the dopamine D2 GPCR. Invitrogen has developed a GeneBLAzer® cell-based assay to monitor activation of this GPCR via a beta-lactamase reporter technology. GeneBLAzer® technology employs a membrane-permeant fluorescence resonance energy transfer (FRET)-based substrate that allows detection of a functional response in live cells. The dual-emission wavelength readout significantly reduces experimental variables. To aid in the identification of possible drug targets for PD, Invitrogen has developed a GeneBLAzer® cell-based assay to monitor activation of the dopamine D2 GPCR via a beta-lactamase reporter technology. GeneBLAzer® technology employs a membrane-permeant fluorescence resonance energy transfer (FRET)-based substrate that allows detection of a functional response in live cells. The dual-emission wavelength readout significantly reduces experimental variables. To aid in the identification of possible drug targets for PD, Invitrogen has developed a GeneBLAzer® cell-based assay to monitor activation of the dopamine D2 GPCR via a beta-lactamase reporter technology. GeneBLAzer® technology employs a membrane-permeant fluorescence resonance energy transfer (FRET)-based substrate that allows detection of a functional response in live cells. The dual-emission wavelength readout significantly reduces experimental variables.

![Figure 1. Schematic of cellular and pathway components involved in Parkinson's disease, along with current therapy approaches.](image)

**Figure 1.** Schematic of cellular and pathway components involved in Parkinson’s disease, along with current therapy approaches.

**Figure 2. Measuring dopamine D2 activation or inhibition using GeneBLAzer® technology.**

(A) GeneBLAzer® D2-Gqo5-NFAT-bla CHO-K1 (Cat. No. K1708) cells were activated by incubation with serial dilutions of pergolide, bromocriptine, or apomorphine and then loaded with LiveBLAzer™-FRET B/G Substrate (Cat. No. K1095). (B) Inhibition of apomorphine-induced activation by perphenazine. GeneBLAzer® D2-Gqo5-NFAT-bla CHO-K1 cells were incubated with serial dilutions of perphenazine prior to stimulation with 18 nM apomorphine. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission ratios were normalized such that 0% is equivalent to the absence of stimulant while 100% equals that observed with (A) 1 µM dopamine or (B) 18 nM apomorphine.
agonists of the dopamine receptor, the GeneBLAzer® D2-Gqo5-NFAT-bla CHO-K1 cell line was developed to monitor activation of the D2 GPCR via a beta-lactamase reporter gene. The cell line contains the stably integrated human dopamine receptor 2 (D2), a beta-lactamase (bla) reporter gene under control of the nuclear factor of activated T cells response element (NFAT), as well as the chimeric G-protein Gqo5 in the CHO-K1 cell line. The results show that D2 receptor agonism can be quantified for the Parkinson’s disease drugs bromocriptine, pergolide, and apomorphine; furthermore, D2 receptor antagonism was readily quantified using apomorphine as the agonist (Figure 2).

Alternatively, this cell line may be used to determine the specificity of receptor agonists and/or antagonists. The SelectScreen® Cell-Based GPCR Profiling Service allows you to not only identify new agonists and/or antagonists for the D2 receptor, but also determine the selectivity of your compounds for the receptor by profiling against a panel of any of our 132 available GPCR cell lines.

LanthaScreen® TR-FRET assay for LRRK2

Mutations in the kinase-encoding gene LRRK2 have been identified in association with PD [1]. At least 20 such mutations have been discovered, scattered across the large, multidomain protein. The most prevalent mutation, G2019S, has been demonstrated to increase the kinase activity of LRRK2. Discovering an LRRK2 inhibitor could lead to new therapeutic agents for treating the disease. Invitrogen has developed a collection of high-throughput screening tools for wild-type LRRK2 and the LRRK2 G2019S mutant. We have generated highly purified and active LRRK2 enzymes and the kinase-dead mutant LRRK2 D1994A (Figure 3), which can be used to screen potential inhibitors in a fully validated LanthaScreen® TR-FRET kinase assay. This assay provides sensitive and specific detection of LRRK2 kinase activity using a peptide substrate derived from ezrin/radixin/moesin (ERM) labeled with fluorescein. Phosphorylation of the peptide recruits a terbium-labeled phosphospecific antibody, resulting in FRET. Due to the TR-FRET readout, the assay is highly resistant to compound interference and is also suitable for evaluating inhibitor potencies (Figure 4).

Let us enable your search for potential mediators of LRRK2 activation by screening your compound libraries using our SelectScreen® Library Screening Service. We can also profile your lead candidates using the SelectScreen® Biochemical Kinase Profiling Service.

Tools for studying potential PD drug targets

Take advantage of Invitrogen’s biochemical and cell-based assays for dopamine D2 and LRRK2, and rely on Invitrogen’s SelectScreen® Service for outsourcing your screening and profiling projects. Learn more about these assays and services at www.invitrogen.com/bp60.

Reference


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Mitochondrial fission and fusion in neurodegeneration

Defects in neuronal mitochondrial function are key indicators in many neurodegenerative diseases, including Alzheimer’s disease, amyotrophic lateral sclerosis, Parkinson’s disease, and ischemic stroke, along with many other disease states outside the nervous system. For example, cytochrome c leakage, imbalance in reactive oxygen and nitrogen species, and activation of apoptotic pathway genes and proteins have all been described in one or more of these diseases.

Shifting the balance to mitochondrial fragmentation

Recent evidence suggests a parallel morphological correlation in which the balance between fission and fusion of the mitochondrial syncytia is disrupted. Nitric oxide, amyloid-β, rotenone, ApoE4 protein, and oxidative and nitrosative stress can all shift the balance from fusion to fission (fragmentation) [1–5] (Figure 1). These same stresses can alter mitochondrial distribution and morphology in cells—possibly via a key motor protein that induces fission [6]. A number of GTPases are critical for fusion events and have also been implicated, with upregulation of their activity protecting against neurodegeneration [7,8]. Simple morphological visualization of mitochondria in live or fixed cells can thus be used as a key phenotypic marker and indicator of stress and as an effective model in understanding inducers of neurological pathologies.

Options for visualizing mitochondria

Invitrogen offers a variety of reagents available for staining mitochondria (Table 1), including MitoTracker® probes (Figure 2), other potential-sensitive mitochondrial probes (e.g., JC-1, JC-9, and tetramethylrhodamine methyl and ethyl esters), mitochondrial-targeted Organelle Lights™ reagents (Figure 3), and primary antibodies for detecting specific proteins involved in oxidative phosphorylation or other mitochondrial pathways. Learn more about mitochondrial staining at www.invitrogen.com/bp60.

References

Figure 1. Changes in mitochondrial morphology. Many cellular events and proteins have been described that can regulate and shift the balance of mitochondrial distribution between hyper-fused (tubular) to hyper-fissioned (fragmented). This figure summarizes some of the recent data from the literature [1–8].
Figure 2. Dye-based mitochondrial labeling. Live bovine pulmonary artery endothelial cells were incubated simultaneously with MitoTracker® Deep Red FM (Cat. No. M22426), LysoTracker® Green DND-26 (Cat. No. L7526), and Hoechst 33342 (Cat. No. H3570).

Figure 3. Fluorescent protein–based mitochondrial labeling. Live HeLa cells were transduced with Organelle Lights™ Lysosomes-GFP (Cat. No. O36228) and Organelle Lights™ Mito-RFP (Cat. No. O36229), then stained with Hoechst 33342 (Cat. No. H3570).

Table 1. Products for visualizing mitochondria.

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<td>20 x 50 µg</td>
<td>M7512</td>
</tr>
<tr>
<td>Image-iT® LIVE Mitochondrial and Nuclear Labeling Kit</td>
<td>M7512 in kit form with nuclear stain; compatible with GFP staining</td>
<td>1 kit</td>
<td>I34154</td>
</tr>
<tr>
<td>MitoTracker® Green FM</td>
<td>Color option to M7512, accumulates in polarized mitochondria where it binds to thiol, and thus is retained if mitochondria are depolarized [9]. Has been successfully used to calculate total mitochondrial mass and has been shown to be retained in fixed cells.</td>
<td>20 x 50 µg</td>
<td>M7514</td>
</tr>
<tr>
<td>MitoTracker® Orange CMTMRos</td>
<td>Color option to M7512, with similar properties</td>
<td>20 x 50 µg</td>
<td>M7510</td>
</tr>
<tr>
<td>MitoTracker® Red FM</td>
<td>Color option to M7512, with similar properties</td>
<td>20 x 50 µg</td>
<td>M22425</td>
</tr>
<tr>
<td>MitoTracker® Deep Red FM</td>
<td>Color option to M7512, with similar properties</td>
<td>20 x 50 µg</td>
<td>M22426</td>
</tr>
<tr>
<td>MitoTracker® Red CM-H2XRos</td>
<td>Reduced form of M7512, fluorescence requires both oxidation and mitochondrial membrane potential</td>
<td>20 x 50 µg</td>
<td>M7513</td>
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<tr>
<td>MitoTracker® Orange CM-H2TMRos</td>
<td>Reduced form of M7510, fluorescence requires both oxidation and mitochondrial membrane potential</td>
<td>20 x 50 µg</td>
<td>M7511</td>
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</table>

| Organelle Lights™ reagents  |                                                                       |          |          |
| Organelle Lights™ Mito-GFP  | BacMam-based fluorescent proteins                                     | 1 kit    | O36210   |
| Organelle Lights™ Mito-OFP  | BacMam-based fluorescent proteins                                     | 1 kit    | O36222   |
| Organelle Lights™ Mito-RFP  | BacMam-based fluorescent proteins                                     | 1 kit    | O36229   |

| Other live-cell dyes to assess mitochondrial physiology |                                                                       |          |          |
| MitoSox™ Red mitochondrial superoxide indicator | Accumulates in polarized mitochondria; functional status indicator | 10 x 50 µg | M36008   |
| Dihydroethidium (hydroethidine) | Cytosolic superoxide indicator; intercalates within DNA on oxidation | 10 x 1 mg | D11347   |
| Image-iT® LIVE Mitochondrial Transition Pore Assay Kit | A live-cell apoptosis assay for visualizing the mitochondrial permeability transition | 1 kit    | I35103   |
| HCS Mitochondrial Health Kit | For high-content imaging of mitotoxicity and cytotoxicity             | 1 kit    | H10295   |
| JC-1 (5,5',6'-tetrachloro-1',3',3'-tetraethylbenzimidazolyl-carbocyanine iodide) | Green at low concentrations; forms red aggregates in healthy, polarized mitochondria | 5 mg | T3168    |
| JC-9 (3,3'-dimethyl-α-naphthoxacarbocyanine iodide) | Functions like JC-1, but baseline green signal more stable over range of depolarization | 5 mg | D22421   |

| Classic mitochondrial stains (accumulate in live, polarized mitochondria, but unlike most MitoTracker® probes, are not retained upon fixation) |                                                                       |          |          |
| Rhodamine 123 | Green-fluorescent sensor of mitochondrial membrane potential | 25 mg | R302     |
| Tetramethylrhodamine, methyl ester, perchlorate (TMRM) | Red-orange–fluorescent sensor of mitochondrial membrane potential | 25 mg | T668     |
| Tetramethylrhodamine, ethyl ester, perchlorate (TMRE) | Red-orange–fluorescent dye | 25 mg | T669     |

| Primary antibodies |                                                                       |          |          |

For antibodies and assays to study mitochondrial proteins and determine their role in disease states or pathways, visit [www.invitrogen.com/bp60](https://www.invitrogen.com/bp60)
Cancer is projected to become the leading cause of death worldwide by 2010 [1], while even more lives are severely disrupted by traumatic surgeries and follow-on drug treatments. While some tumors are toxic in situ, most morbidity is associated with cancer cell–associated degradation of the basement membrane and subsequent extravasation to spread throughout the body. Here we provide an overview of Invitrogen’s Molecular Probes® reagents that can be used for the study of cancer metastasis.

Breaching boundaries
A variety of normal cellular processes involve the crossing of cell and tissue borders or boundaries, collectively termed basement membranes or extracellular matrices. For example, macrophages undergo a cascade of adhesive events and selective extracellular matrix (ECM) degradation to depart from the blood system and invade infected tissues. A similar mechanism has been characterized for endothelial cells during the generation of neovessels.

Many of the molecular strategies used in normal cell migration are also employed by tumor cells during the process of metastasis, and therapeutic approaches have focused on blocking these pathways. The process of metastasis and accompanying neovessel formation can be studied using fluorescent dyes and natural protein substrates that facilitate visualization of cell movement and the selective degradation of ECM components constituting tissue boundaries.

**Visualizing tumor metastasis**

**CELLTRACKER™ DYES, DQ™ COLLAGEN, AND GELTREX™ MATRIX.**

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**CellTracker™ dyes—tracking tumor cell migration**

Molecular Probes® CellTracker™ (thiol-reactive) and most CellTrace™ (amine-reactive) dyes freely diffuse through the membranes of live cells and once inside, the action of intracellular esterase converts them to fluorescent forms that survive fixation. Cells labeled in this manner remain viable and fluorescent for days to weeks, even after 5–6 divisions. For cell migration studies, these dyes can be used to follow cells as they migrate through tissue, either artificial or real, and can be multiplexed with functional probes (such as DQ™ collagen, discussed below) for the simultaneous detection of cell migration and ECM degradation. The Sloane lab and colleagues have used CellTracker™ and CellTrace™ dyes extensively [2–5] to visualize the migration of tumor cells through three-dimensional (3D) extracellular matrices, their interaction with stromal fibroblasts, and the simultaneous digestion of the ECM protein collagen IV (Figure 1).

**DQ™ collagen—visualizing tumor-associated proteolysis**

A variety of proteases from tumor cells and tumor-associated cells are critical to the process of metastasis; these proteases facilitate the breakdown of various ECM components during cell migration. The fluorescein conjugate DQ™ collagen can be used to directly monitor...
collagenase activity of tumor cells. This heavily conjugated collagen substrate readily self-quenches, but quenching is relieved upon hydrolysis to single dye–labeled peptides.

DQ™ collagen can be used in live-cell studies [2] (Figure 1) or for in situ zymography on unfixed cryosections [6]. When multiplexed with CellTracker™ dyes, the simultaneous visualization of cell migration and proteolysis can be accomplished, under conditions that mimic the in vivo tumor architecture and its environment (using Geltrex™ matrix, discussed below). Figure 2 demonstrates the experimental setup used for imaging CellTracker™-labeled tumor cell migration through a DQ™ collagen substrate suspended in a 3D basement membrane matrix.

DQ™ collagen assays are ideal for the identification of inhibitors of tumor proteolysis. Sameni and colleagues [4] recently demonstrated the use of DQ™ collagen IV to visualize inhibition of matrix metalloproteinases (MMPs), cysteine cathepsins, and serine proteases in cocultures of breast carcinoma cells and fibroblasts (Figure 3).

**Geltrex™ matrix—mimicking in vivo conditions**

Geltrex™ matrix is a soluble form of basement membrane extract purified from continuous sheets of specialized ECM that form an interface between Engelbreth-Holm-Swarm (EHS) tumor cells. Like naturally occurring basement membrane, Geltrex™ matrix not only supports cells and cell layers but also plays an essential role in tissue organization that affects cell adhesion, migration, proliferation, and differentiation. Because it mimics in vivo conditions, Geltrex™ matrix provides an ideal setting for in vitro metastasis assays when combined with DQ™ substrates (Figure 2).

**Figure 2. Imaging tumor cell migration in vitro.** Tumor cells are labeled with CellTracker™ dye and migrate through DQ™ collagen substrate suspended in three-dimensional matrix gel. Green fluorescence indicates the region of collagen degradation by secreted proteinases. In this schematic, cells are labeled orange with CellTracker™ Orange dye, and nuclei are stained blue with Hoechst 33342.

**Figure 3. Inhibition of proteolysis of DQ™ collagen IV.** Cocultures of BT549 breast carcinoma cells and WS-12Ti fibroblasts (prelabeled with CellTrace™ Far Red, Cat. No. C34553) were treated with inhibitors of MMPs (GM6001), cysteine cathepsins (CA074/CA0Me), and serine proteases (aprotinin). The bar above each image represents fluorescence intensity per cell. DQ™ collagen degradation appears green, and nuclei were stained blue with Hoechst. With kind permission from Springer Science+Business Media: Sameni M et al. (2009) Imaging and quantifying the dynamics of tumor-associated proteolysis. Clin Exp Metastasis 26:299–309, Figure 3.

**More reagents for tumor metastasis studies**

To find all available assays for visualizing tumor metastasis including CellTracker™ dyes, Qdot® nanocrystals, and fluorescent protein–based Cellular Lights™ reagents, visit [www.invitrogen.com/bp60](http://www.invitrogen.com/bp60).

**References**


**Product**|
---|
DQ™ collagen, type IV from human placenta, fluorescein conjugate | 1 mg | D12052
DQ™ collagen, type I from bovine skin, fluorescein conjugate | 1 mg | D12060
Geltrex™ Reduced Growth Factor Basement Membrane Matrix | 1 mL | 12760013
CellTracker™ Blue CMAC | 5 mg | C2110
CellTracker™ Green CMFDA | 20 x 50 µg | C7025
CellTracker™ Orange CMTMR | 1 mg | C2927
CellTracker™ Orange CMRA | 20 x 50 µg | C34551
CellTracker™ Red CMTPX | 20 x 50 µg | C34552
Vybrant® CFDA SE Cell Tracer Kit | 1 kit | V12883
CellTrace™ Far Red DDAO | 20 x 50 µg | C34553
Innovative solutions for ion channel research
CELL-BASED AND BIOCHEMICAL ASSAYS FOR ION CHANNEL ACTIVITY.

Ion channels are transmembrane proteins that facilitate ion flow across cells, thereby controlling the membrane potential and membrane excitability. Sodium ions are concentrated outside cells, and potassium ions are concentrated inside cells; there is also a charge gradient from net positive in the extracellular fluid to negative in the cytosol. Ion-selective channels open in response to stimuli and conduct electrical currents across cells in accordance with these gradients to regulate many important processes in biology.

An estimated 400 ion channel genes exist [1]. Functional diversity is increased by the formation of heteromeric channels from multisubunit complexes and the interaction of specific channels with accessory proteins [2], generating a very large number of functional ion channel signatures. Study of these ionic currents in voltage clamp experiments is a major goal of heterologous expression, and a major challenge where subunit stoichiometry is critical in cellular function of the complex.

Ion channels are critical to a number of cellular functions and processes. They mediate information transfer and control cellular homeostasis. Ion channels underlie nerve impulses and signal transmission across synapses and make up the quantal units of communication in the central nervous system. In addition, they are involved in a wide range of biological processes that require rapid cellular changes, such as muscle contraction, epithelial transport of nutrients and ions, T cell activation, and hormonal release. As a consequence, ion channel dysfunctions have been associated with many disease states [1,3].

Despite the large number of potential targets and their physiological importance, ion channels remain relatively unexploited as pharmaceutical targets. This has been due to challenges in the development of assays and cellular models suitable for drug discovery efforts [4,5]. Methods for the detection of ion channel activity appropriate for modern drug discovery requirements have lagged behind those of other target classes. Recently, however, tools for measuring ligand displacement, membrane potential, and ion flux and automated patch instruments have enabled high-throughput screening of ion channel targets.

A second challenge is generating the cellular models required for experimentation and screening. The traditional approach of using stable cell lines has been difficult due to toxicity, clonal drift with passage, and other factors. Investigators are therefore increasingly turning to inducible or transient expression systems.

Molecular Probes has been a leader in the development of reagents for cell-based assays, including those for ion channels. Fluo-4 may be the most widely used reagent for studies of calcium flux, either via calcium ion channels or as a second messenger in G-protein–coupled receptor activation. Recently, we introduced a novel suite of reagents to enable holistic studies of ion channel activity in a variety of contexts with cellular and biochemical assays. Broadly, these can be classified into two categories: sensors to measure ion channel activity in high throughput and transient expression of ion channel genes.

Potassium channels
Potassium ion channels are represented by more than 80 genes in human cells [6]. Most potassium channels have a tetrameric structure. Both homotetrameric and heterotetrameric complexes exist in nature, generating a wealth of pharmacological, structural, and functional diversity. Potassium channels are the most widely distributed type of ion channel and are virtually ubiquitous in mammalian cell types, controlling a wide variety of functions. In excitable cells such as neurons, muscle, and vascular cells, they shape action potential duration and set the resting membrane potential, affecting every other voltage-gated channel type in the membrane. Because potassium ion channels regulate the action potential duration in cardiac muscle, pharmacological blockage or malfunction of their activity may cause life-threatening arrhythmias and other cardiovascular diseases. Potassium channel activity also regulates cellular processes such as the secretion of hormones, and their malfunction in this context has been linked to several disease states such as diabetes, cardiac disease, immune disorders, and hypertension [1,7].
Sodium channels

Sodium channels are classified according to whether the trigger that opens the channel is voltage- or ligand-gated [8]. Voltage-gated channels are responsible for action potential initiation and propagation in most excitable cells, including nerve, cardiac, muscle, and neuroendocrine cell types. This family of sodium channels has nine known members, with amino acid identity >90% in the pore, voltage sensor, transmembrane, and extracellular loop regions [8]. These proteins are formed from a large pore-forming α subunit (~220–260 kDa) that associates with regulatory β subunits and other factors to regulate fast electrical events in excitatory tissues of the central and peripheral nervous system. Their size and extensive homology have made recombinant α subunits particularly challenging to express stably. All pharmacological agents that act on sodium channels have well-described receptor sites on the α subunits, but with only subtle differences within the family, drugs that can discriminate subtypes have been extremely difficult to identify.

Chloride channels

Chloride channels are also involved in a very broad range of cellular processes, so defects in their activity are typically severe, causing cystic fibrosis, Bartter syndrome, myotonia congenita, and neuronal degeneration [9]. A number of drugs for the treatment of insomnia, anxiety, convulsions, and mood disorders also target neuronal chloride channels [10].

Types of assays for ion channel activity

Traditional patch-clamp measurements of ion channel activity are too expensive and labor-intensive for large-scale screening. Membrane potential dyes can report transmembrane voltage changes when channels are opened or closed, and have supported some high-throughput screening (HTS), though these dyes do not discriminate ion-selective activity. Fluorescent calcium indicators such as fluo-4 allow the detection of intracellular calcium and therefore assay ion-specific activity, which has already been successful for HTS of a handful of calcium ion channels.

FluxOR™ potassium channel assays

The FluxOR™ assay is a universal potassium ion channel assay that produces relevant pharmacological data using standard fluorescence microplate readers. While ion channel activity is most accurately measured with patch-clamp techniques, even automated systems are too labor-intensive for most screening efforts. The FluxOR™ assay is an easy-to-use, robust HTS assay that can measure the activity of any potassium-selective ion channel.

The basis for the FluxOR™ assay is the bright fluorescence of the FluxOR™ dye upon binding thallium ions, which serve as surrogate ions for potassium. Drugs that inhibit potassium channels diminish the response, while drugs that activate the channels increase it. The fluorescent signal is proportional to potassium channel activity (Figure 1). FluxOR™ dye does not have significant affinity for calcium, which may interfere with measurements using other thallium-sensitive dyes, and off-target effects from other ion channels in the cells do not interfere with the signal. This specificity reduces the number of false positives in drug screening campaigns, where millions of compounds have to be tested for activity at a specific ion channel. Because the FluxOR™ assay is cell based, the channels are observed in an intact biological system, which means the pharmacology will be more closely related to that of assays downstream of the primary screening. The FluxOR™ platform also reports the activity of allosteric or modulatory compounds that control potassium ion channel activity, not just those that close the pore.

Assays based on voltage sensor probes (VSPs)

Another method for measuring ion channel activity in microplate format uses membrane potential dyes. All active ion channels affect cellular membrane potential, and fluorescent dye kits have been developed that report when channels of any type are opened or closed. VSPs report the potential across the cell membrane via a fluorescence resonance energy transfer (FRET) principle. VSP kits combine an
outer membrane–bound donor and a mobile acceptor that traverses the membrane in response to changes in membrane potential. The FRET-based measurement is restricted to the plasma membrane and is therefore more relevant than non-FRET approaches that measure signals throughout the cell.

While sodium-specific sensors suitable for HTS assays are lacking, VSPs faithfully respond to voltage-gated sodium channel activity in a variety of cellular models [11,12]. Sodium ion movement through open channels causes membrane depolarization that can be measured with a large and reproducible signal. Benefits include rapid, high-throughput, high-sensitivity detection, minimal well-to-well variation, and applicability to any target that changes the membrane potential.

**Premo™ Halide Sensor assays**

The Premo™ Halide Sensor combines fluorescence detection with BacMam technology, which uses an insect cell virus (baculovirus) to efficiently deliver and express genes in mammalian cells [13,14] (Figure 2). The sensor uses a halide-sensitive Yellow Fluorescent Protein (YFP) variant to detect changes in local halide concentration.

The assay is based on the permeability of chloride channels to iodide, the strong gradient across the plasma membrane of this ion, and higher quenching of fluorescence by iodide than chloride. YFP can be detected using standard GFP/FITC or YFP filter sets. Iodide-sensitive fluorescent proteins have been successfully used in HTS applications to screen activators of the cystic fibrosis transmembrane regulator, calcium-activated chloride channels, and GABA A receptor chloride channels [15,16]. Use of a YFP-based biosensor for chloride channel activity in a drug discovery setting has recently been reviewed [9].

Premo™ Halide Sensor assays enable real-time kinetic measurements, allowing assessment of the modulation of chloride channel conductance with previously unavailable sensitivity, reliability, and precision.

**BacMam-mediated ion channel expression**

Cell-based assays have typically been based on the stable expression of a target in a transformed cell line. For some protein classes, including ion channels, constitutive expression is frequently associated with cytotoxicity and clonal drift upon passage, leading to variable experimental results. Researchers have therefore begun to explore transient expression approaches, and one of the most promising is BacMam technology [13,14]. BacMam technology has successfully driven discovery campaigns at diverse targets, including ion channels and transporters [17–20]. As mammalian cells do not support baculovirus replication, transduction is very well tolerated and generally lacks visible cytopathic effects; baculoviruses are considered safe research reagents, with a BSL 1 classification.

For multicomponent systems that require stoichiometric expression, the ability to control transient expression by simply varying the dose enables screening in a wide range of cell types (Figure 3). Gene expression is unaffected by freezing of transduced cells, so it is easy to generate a large batch of assay-ready cells. The BacMam system thus provides flexibility and faster assay development timelines.

These reagents have proven particularly useful in discovery using automated patch-clamp platforms [21]. The ability to simultaneously deliver...
more than one BacMam ion channel to a cell enables interrogation of multiple channels, which increases throughput and data quality (Figure 4).

Holistic approach to ion channel interrogation

The ability to combine target expression and robust assays opens new research avenues. Fluo-4 was a breakthrough for high-sensitivity calcium detection in live cells and propelled G-protein second-messenger detection and calcium channel activity research to a new level. The tools described here help fill a similar need for other ion channel targets. For example, BacMam technology, which provides transient expression of potassium channels, and the FluxOR™ assay [22], which measures potassium channel activity, make a powerful combination [23]. This combination makes research avenues. Fluo-4 was a breakthrough for high-sensitivity calcium activity, make a powerful combination [23]. This combination makes research, visit www.invitrogen.com/bp60.

Figure 4. Automated patch-clamp measurement of a U2OS cell coexpressing BacMam hERG (Kv11.1) and Nav1.5 ion channels. (A) Lower trace shows the applied voltage. The cells were held at −100 mV and a tail current applied (blue trace) to measure the outward hERG-mediated current (upper trace). A second 0.5 ms step (red trace) was made to capture the peak inward current carried by Nav1.5, shown on an expanded timescale in (B).

References

Resources for cytokine research
ANTIBODIES, RECOMBINANT PROTEINS, ELISAS, AND LUMINEX® KITS.

Cytokines are secreted cell signaling proteins or peptides that regulate many aspects of the immune system. Approaches to studying cytokine function are varied and include the use of antibodies, recombinant proteins, and ELISA and Luminex® kits. Invitrogen offers a diverse array of products for investigating cytokines, their cell surface receptors, and downstream signaling pathways for a variety of applications.

Cytokine antibodies
Invitrogen’s extensive portfolio of cytokine antibodies, including phosphospecific and fluorescently labeled antibodies, are validated for multiple applications including western blotting, ELISAs, immunocytochemistry, immunohistochemistry, and flow cytometry. Find antibodies for your cytokine or downstream pathway of interest at www.invitrogen.com/bp60.

Recombinant cytokine proteins
Invitrogen’s recombinant proteins and growth factors exhibit high purity and potent biological activity. Rigorous in-house testing of biological activity includes assays that measure cell proliferation, cytotoxicity, chemotaxis, calcium flux, secondary cytokine upregulation, and induction of surface antigen expression (Figure 1). Find recombinant proteins for your cytokine research at www.invitrogen.com/bp60.

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Invitrogen has a wide menu of ELISA kits with world class sensitivity, proven specificity, and consistent performance. ELISA and phospho-ELISA™ kits can be used for precise quantitation of expressed cytokines, with high sensitivity and specificity (Figure 2). Learn more at www.invitrogen.com/bp60.

Luminex® assays for multiplex cytokine analysis
Invitrogen offers extracellular marker assays for accurate quantitation of cytokines, chemokines, and growth factors. Available as singleplex or multiplex kits, the assays can detect picogram levels of protein and can be run in ~4 hours. Find our full range of Luminex® assays at www.invitrogen.com/bp60.

**Figure 1.** Biological activity of recombinant human IL-8. Chemotaxis assay using human polymorphonuclear leukocytes (PMNs) fluorescently labeled with BCECF-AM. Serial dilutions of IL-8 (Cat. No. PHC0884) were incubated with PMNs in a chemotaxis chamber. Fluorescence was measured in a fluorometer using excitation/emission of 485/530 nm.

**Figure 2.** IFN-γ activation of STAT1. HeLa cells were treated with recombinant human IFN-γ (Cat. No. PHC4031) at varying concentrations for 20 min, then lysed. Phosphorylation of tyrosine 701 increases with dosage of IFN-γ as measured by the STAT1 [pY701] phosophoELISA™ kit (Cat. No. KHO0271). The results correlated well with western blot analysis of the same samples.
**CLICK-IT® EDU IMAGING AND HCS KITS**

The fastest, easiest, and most accurate method for detecting cell proliferation via nascent DNA synthesis now offers even more advantages. The Click-it® EDU Imaging and HCS kits have been reformulated to further streamline the assay protocol, and now include a wash buffer to maximize the signal-to-noise ratio. Learn more at www.invitrogen.com/bp60.

**AbC™ ANTI-RAT/HAMSTER BEAD KIT**

Compensation is crucial for accurate multicolor experimentation. Because many antigens are not highly expressed, cells may not always generate the fluorescence signal needed for correcting spectral overlap, and dimly stained cells could lead to improper compensation settings. The AbC™ Anti-Rat/Hamster Bead Kit, for use with conjugates of any rat or hamster IgG, overcomes this problem. Each kit provides polystyrene microspheres that are either coated with capture antibody (positive) or uncoated (negative). After mixing with a fluorophore-conjugated rat or hamster antibody, the two components provide distinct positively and negatively stained bead populations, which can then be used to set compensation. Learn more at www.invitrogen.com/bp60.

**ACCURATE CELL IDENTIFICATION FOR HCS PLATFORMS**

Molecular Probes® HCS CellMask™ and HCS NuclearMask™ stains provide reliable cell delineation with a choice of fluorescent colors ranging from blue to deep red. HCS CellMask™ stains label the whole cell, including cytoplasm and nucleus, and are applied to cells immediately after fixation and permeabilization or after antibody labeling. The versatile HCS NuclearMask™ stains measure DNA content in addition to enabling robust cell demarcation of live and formaldehyde-fixed cells. Learn more about tools for high-content imaging and analysis at www.invitrogen.com/bp60.

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Invitrogen has a rapidly expanding menu of directly conjugated primary antibodies with human and mouse reactivity that are available in a variety of colors to help maximize multicolor flow cytometric analysis. These antibody conjugates can help answer complex biological questions relating to cancer, immunology, stem cell research, cell signaling, and studies of T, B, and NK cells, and more. In addition, these conjugates allow simultaneous measurement of different specificities to help reveal more information about each individual cell with less time and less sample. View all of our new antibodies at www.invitrogen.com/bp60.

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Recently published

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

Neuroscience: In situ optical recording of action potentials from mouse nerve terminals. The quest to understand neural processing at its most fundamental level requires development of optical techniques capable of discrete detection of action potentials at single-cell resolution in three-dimensional tissue preparations to augment invasive electrode array techniques with limited spatial resolution. Researchers from the University of Pennsylvania have advanced this quest using two-photon excitation of the potentiometric styryl dye di-3-ANEPPDHQ in nerve terminals of intact mouse neurohypophysial preparations. They demonstrated single-trial recordings of action potentials with signal-to-noise ratios of 5:1 and fractional fluorescence changes of up to 10%. Because the potentiometric response of fluorescent dyes is highly specimen-dependent, careful in situ optimization of the two-photon excitation wavelength was required to obtain this level of performance. The selection of di-3-ANEPPDHQ for these measurements was based on its relatively low phototoxicity and its resistance to internalization from the plasma membrane, which results in diminished background fluorescence and therefore increased signal-to-noise.


Stem cells: Using proteins to induce pluripotency in somatic cells. The reprogramming of somatic cells into pluripotent stem cells has previously been achieved by transforming those cells with genetic sequences that code for factors necessary for the reprogramming. However, the introduction of exogenous genetic material carries the risk of unpredictable genetic modifications of the target cell genome. In their recent report, Zhou and colleagues describe the generation of induced pluripotent stem cells from mouse embryonic fibroblast cells following transduction with the recombinant reprogramming proteins Oct4, Sox2, Klf4, and c-Myc containing a protein transduction domain fused at the C-terminus. Pluripotency of the resulting cells was demonstrated through immunolabeling experiments using Alexa Fluor® dye-labeled antibodies directed toward known pluripotency markers. These protein-induced pluripotent stem cells (piPSPs) were stably and homogeneously expanded for more than 30 passages; the cells were morphologically indistinguishable from mouse embryonic stem cells (mESCs), and were observed to efficiently incorporate and differentiate in vivo.


Astrobiology: Amino acid detection in Martian analogue samples using Pacific Blue™ dye. The search for evidence of life on other planets has centered on the detection of amino acids, due to their ubiquitous presence in terrestrial organisms. For an upcoming Mars mission, the European Space Agency has developed a portable capillary electrophoresis instrument—the Mars Organic Analyzer (MOA)—for the in situ detection and analysis of bioamines. Utilizing the amine-reactive succinimidyl ester form of Pacific Blue™ dye for fluorescence-based detection, the development team has demonstrated successful detection, and subsequent identification and chirality analysis, of amino acids at levels as low as 75 pM (sub–parts-per-trillion) in prepared samples. This represents a 200-fold increase in sensitivity as compared to detection based on fluorescamine; the team used this greater sensitivity for the successful analysis of amino acids at the parts-per-billion level in terrestrial (Atacama Desert) and astrobiological (Murchison meteorite) samples.


Evolutionary biology: Fluorescence analysis of the evolution of neural structures necessary for vocalization. In an effort to establish the ancestral origin of vocal communication, Bass and colleagues have made extensive use of Alexa Fluor® dye–labeled probes, including Alexa Fluor® 488 dextran amine and Alexa Fluor® 546 dextran amine, to map the development of vocal neural networks in larval ray-finned fish. Their results demonstrate vocal network development in a hindbrain-spinal region; taxonomic comparison showed this development pattern to be consistent with that observed in studies of multiple lineages of vocal vertebrates. Their results suggest the origin of a vocal network in a common ancestor of ray-finned and lobe-finned fish; further study on the highly conserved nature of this vocal network region could significantly push back the common origin of this neural basis for vocalized social signaling.


The publications summarized here are just a few of the recent additions to the 59,000+ references describing applications of Invitrogen™ products in our searchable bibliography database. Visit www.invitrogen.com/support to search for citations by product.