CELLestial™ Assay Kits for Live Cell Analysis

Cell Structure and Organelle Markers
Membrane Potential Detection
Reactive Oxygen/Nitrogen Species Detection
Calcium Indicators
Cell Death, Apoptosis, Autophagy

incorporating

www.enzolifesciences.com

Based on a very substantial intellectual property portfolio, Enzo Life Sciences, Inc. is a major developer and provider of labeling and detection technologies across research and diagnostic markets. A strong portfolio of labeling probes and dyes provides life science environments with tools for target identification and validation, and high content analysis via gene expression analysis, nucleic acid detection, protein biochemistry and detection, molecular biology, and cellular analysis.

- Genomic Analysis
- Post-translational Modification
- Cancer & Immunology
- Cellular Analysis
- Signal Transduction
- Drug Discovery

In addition to our wide range of catalog products, a complementary range of highly specialized custom services are also offered to provide tailor-made solutions for researchers. These include small molecule organic synthesis, custom-labeled FISH probes, peptide synthesis, protein expression, and antibody production.
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Introduction

Novel Cell Functional Assays for Toxicity Testing of Pre-clinical Drug Candidates

The effect of drugs or other xenobiotics (e.g. toxins, environmental contaminants) upon living organisms depends very much upon their concentration at the site(s) of their action [1]. Cationic drugs in particular often exhibit large apparent volumes of distribution, which is consistent with various forms of sequestration within cells and tissues. This form of drug uptake can occur in intact cell types that are not necessarily specialized for the handling of xenobiotics. The drugs are sequestered into cells by a variety of metabolically-driven, but receptor-independent means including mitochondrial membrane potential-driven concentration, nuclear concentration via DNA affinity and vacuolar-ATPase-driven trapping into lysosomes that subsequently swell by an osmotic mechanism. Drug concentration within cells may arise from the innate membrane permeability of the uncharged forms of these drugs, as well as by means of specific transporters (organic cation transporters, choline transporters, etc.). Such drug sequestration can contribute to overall toxicity, as well as prolong the duration of a drug’s action.

Toxicity continues to be responsible for more than 30% of compound attrition during the drug development process and remains one of the major causes for drugs being withdrawn after their approval [2]. The US Food and Drug Administration (FDA) guidance of “Safety Testing of Drug Metabolites” published in February, 2008 encourages evaluation of metabolite toxicity early in the drug development process [3]. Enzo Life Sciences is developing a variety of innovative assays that are potentially applicable to in vitro toxicology testing during preclinical and clinical drug development. These assays provide robust and rapid in vitro toxicity screening approaches to identify compound liabilities and STR (structure toxicity relationship) associated with new chemical entities (NCEs), aiding in selection and optimization of NCEs in the early stages of drug discovery. The results obtained from multiple assays and dose-response profiles allow better understanding of the potential toxicity of compounds and thus facilitate selection of compounds with a higher probability of success.

The panel of fluorescence-based live cell assays are designed to assess the impact of xenobiotics on overall cell function, with particular emphasis on the plasma membrane, lysosomal, mitochondrial and nuclear compartments. Many of the assays have been optimized for analysis by flow cytometry, while others are geared towards microplate-based cytometry. The semi-automated multi-well cell-based assay workflow provides a rapid and quantitative high-throughput approach for determining drug- or toxic agent-induced live cell response, offering throughput advantages relative to methods based upon electron microscopy, fluorescence microscopy or flow cytometry. Early secondary screening of candidate drugs for potentially adverse cell activity in the drug discovery phase could predict later risks in drug development arising from drug safety issues. Such a screening approach can aid in selecting the best candidate compounds for further drug development efforts, as well as provide preliminary benchmarking of dosing limits in preclinical toxicity studies.
Enzo Life Sciences provides a variety of tools to help pharmaceutical and biotech companies optimize their pre-clinical and clinical drug development programs through early lead drug identification, lead candidate selection, predictive toxicology and compound characterization. The various cell-based assays are summarized schematically in Figure 1. Overall, these assays may be broadly classified into four main categories:

- **Death pathway assays:** Cell-based assays for monitoring compound effects on apoptosis, autophagy and necrosis. Includes monitoring of annexin V redistribution, nuclear condensation, autophagosome generation and loss of plasma membrane integrity.

- **Oxidative stress, antioxidants & scavenger assays:** Cell-based assays for monitoring compound effects on reactive oxygen species, superoxide, nitric oxide and peroxynitrite.

- **Cell cycle and transcriptional arrest assays:** Cell-based assays for monitoring compound effects on cell cycle dynamics, ribosome biogenesis, inhibition of transcription and cellular stress.

- **Toxicology assays:** Cell-based assays for monitoring compound effects on the function and viability of human and rodent cell lines. Includes monitoring cell viability, cell proliferation/cytotoxicity, lysosome expansion and mitochondrial activity.

**LITERATURE REFERENCES:**


Nuclear-ID™ Red Cell Cycle Kit
GFP-Certified™ for flow cytometry

- Measures DNA content in live, permeabilized or fixed cells
- Monitor changes in cell cycle dynamics arising from drug treatment or other perturbations
- True multiplexed capability with GFP and other green fluorescent probes used in immunphenotypic analyses
- Easy staining protocol, simply add the dye and analyze by flow cytometry
- Performance validated using a wide range of cell densities
- Nuclear-ID™ Red dye specific signals (Excitation 568nm, Emission 637nm)

Nuclear-ID™ Red Cell Cycle Analysis Kit determines the percentage of cells in a given sample in G0/G1, S and G2/M phases, and quantifies cells in the sub-G0 phase. Perform DNA studies in live, permeabilized and fixed cells for normal cell lines and cell lines exhibiting multiple ploidy levels. Nuclear-ID™ Red Reagent is validated for live cell assay densities ranging from 1 x 10⁵ to 5 x 10⁵ cells/ml. This kit provides a convenient approach to analyze induction and inhibition of cell cycle progression by flow cytometry. Nocodazole, a control cell cycle perturbation agent is provided for monitoring changes in cell cycle dynamics.

**FIGURE 1:** Drug treatments with live cells inhibit cell cycle progression at different phases.

**FIGURE 2:** Three-dimensional reconstruction of the spatial relationship between the green fluorescent protein-expressing (GFP-expressing) mitochondria and the nucleus using a structured illumination method, as implemented with the ApoTome from Carl Zeiss, Inc. This imaging method enabled creation of optical sections through the nucleus using a conventional fluorescence microscope, for improved resolution along the optical axis. The optical sections were then used to create a 3-D reconstruction of the nucleus, enabling the GFP-expressing mitochondria to be displayed in their proper spatial context.
Nuclear-ID™ Green Cell Cycle Kit
for flow cytometry

ENZ-51014 100 Assays

KIT CONTAINS:
100µl, Nuclear-ID™ Green Cell Cycle Detection Reagent
10µg, Nocodazole Control
15µl, 10X Assay Buffer

- Measures DNA content in live, permeabilized or fixed cells
- Performance validated with a wide range of cell densities
- Excited using common 488nm laser source, generating a green ~530nm emission
- Easy staining protocol, simply add dye and analyze by flow cytometry
- Nuclear-ID™ Green dye specific signals (Excitation 503nm, Emission 531nm)

Nuclear-ID™ Green Cell Cycle Analysis Kit determines the percentage of cells in a given sample in G₀/G₁, S and G₂/M phases, and quantifies cells in the sub-G₀ phase. Perform DNA studies in live, permeabilized and fixed cells, using normal cell lines and cell lines exhibiting multiple ploidy levels. Nocodazole, a control cell cycle perturbation agent is provided for monitoring changes in cell cycle dynamics. Live-cell studies include cellular DNA content and cell cycle distribution for the detection of variations in growth patterns, for monitoring apoptosis, and for evaluating tumor cell behavior and suppressor gene mechanisms.

FIGURE 1: Performance is validated for a wide range of cell densities.

FIGURE 2: DNA detection in live HeLa cells using Nuclear-ID™ Green dye and wide-field fluorescence microscopy.
Nucleolar-ID™ Green Detection Kit
for microscopy

- Specifically designed for visualization of nucleoli in live cells
- Compatible with common live-cell nuclear counter-stains including Nuclear-ID™ Red DNA Stain
- High resistance to photobleaching and concentration quenching, ensuring strong, consistent fluorescence signal, even after extended viewing
- Demonstrates appropriate response to treatment with well-characterized nucleolar-perturbation agents
- Nucleolar-ID™ Green dye specific signals (Excitation 450nm, Emission 481nm)

Nucleolar-ID™ Green Detection Kit is specifically designed to stain nucleoli in living cells and observe dynamic changes in the nucleolus. Nucleolar-ID™ Green dye doesn’t co-emit in the Texas Red region of the light spectrum. Thus it can be used in conjunction with a red nuclear stain to examine the changes in this organelle in relation to the organization of the DNA within the cell nucleus. Monitor impaired ribosome biogenesis, inhibition of transcription, cell cycle dynamics and cellular stress, as well as the distribution, trafficking and dynamics of nucleolar proteins and dispersal of viral proteins and potentially, as an aid in identifying cancer cells. Actinomycin D, a nucleolar perturbation control agent, is provided for monitoring changes in nucleolar dynamics.

FIGURE 1: Nucleolar-ID™ Green dye stained live U2OS cells counterstained with Nuclear-ID™ Red (A), Draq5™(B), Vybrant® DyeCycle™ Ruby (C), and Hoechst 33342 (D). Nucleoli appear as green structures contrasted against the selected nuclear counterstain by fluorescence microscopy. As an RNA stain, the Nucleolar-ID™ Green dye displays some cytoplasmic staining as well, but the nuclear counterstain facilitates unambiguous identification of the nucleoli as green fluorescence signal within the confines of the highlighted nuclear fluorescence signal.

FIGURE 2: Cells stained with Nucleolar-ID™ Green Detection Reagent show maximal fluorescence signal within the nucleoli and faint fluorescence throughout the nucleus and cytoplasm, as shown in panel A. When the cells are treated with low doses of actinomycin D, loss of nucleolar staining is observed, as shown in panel B. Counterstaining with Nuclear-ID™ Red Detection Reagent facilitates highlighting the nucleoli relative to the weak cytoplasmic staining.

ENZ-51009  500 Assays

KIT CONTAINS:
50µl, Nucleolar-ID™ Green Detection Reagent
50µl, Actinomycin D Control
125µg, 10X Assay Buffer
Total Nuclear-ID™ Green/Red Nucleolar/Nuclear Detection Kit for microscopy and flow cytometry

ENZ-51006  500 Assays

KIT CONTAINS:
50µl, Nucleolar-ID™ Green Detection Reagent
50µl, Nuclear-ID™ Red Detection Reagent
125µg, Actinomycin D Control
15ml, 10X Assay Buffer

- Simultaneous staining of both the nucleolus and the nucleus of live cells
- Highly resistant to photobleaching and concentration quenching
- Monitor drug-induced changes in the nucleolus
- Spectrally pure green signal allows multiplexing
- Nuclear counter-stain facilitates analysis
- Nuclear-ID™ Red Detection Reagent (Excitation 568nm, Emission 637nm)
- Nucleolar-ID™ Green Detection Reagent (Excitation 450nm, Emission 481nm)

Nuclear-ID™ Green/Red Kit contains two proprietary dyes suitable for simultaneous staining and visualizing of nucleoli and nuclei in live cells. Nucleolar-ID Green™ dye doesn’t co-emit in the Texas Red region of the light spectrum allowing multiplex capability with orange or red dyes. Examine nucleolar dynamic changes in intracellular distribution, trafficking and localization arising from biological processes and monitor impaired dynamics of nucleolar proteins, the distribution of viral proteins, and potentially facilitate identifying cancer cells. A nucleolus perturbation control agent, actinomycin D, is provided for monitoring changes in nucleolar dynamics.

FIGURE 1: Live U2OS cells (A) and HeLa cells (B) stained using the red/green dual fluorescence detection method. The proprietary dyes exhibit minimal spectral overlap with one another, high signal intensity and low photobleaching. The green dye shows maximal fluorescence signal within the nucleoli, and faint fluorescence throughout the nucleus. Weak fluorescence is also observed throughout the cytoplasm, predominantly associated with mitochondria. The red dye maximally stains the DNA in the cell nucleus. The number of nucleoli in different mammalian cell types varied and nucleoli were of different sizes as well. There appeared to be an inverse relationship between the size and number of nucleoli in mammalian cells.

FIGURE 2: Three-dimensional reconstruction of the spatial relationship between the nucleoli and the nucleus using a structured illumination method, as implemented with the ApoTome from Carl Zeiss, Inc. From a hardware perspective, the ApoTome consists of a slider, which inserts into the fluorescence beam path at the plane of the field diaphragm, and a control box to operate the slider. This imaging method enabled creation of optical sections through the nucleus using a conventional fluorescence microscope, for improved resolution along the optical axis. The optical sections were then used to create a 3-D reconstruction of the nucleus, enabling the nucleoli to be displayed in their proper spatial context relative to the body of the nucleus.
Lyso-ID™ Red Detection Kit
GFP-Certified™ for microscopy

- Novel acidic organelle-selective dye for live cell imaging of lysosomes
- Lyso-ID™ Red dye highly phospholipidosis as well as autophagosome accumulation as punctuate signal
- Highly resistant to photobleaching and concentration quenching
- Long wavelength red emission easily multiplexes with common fluorescent dyes (coumarin, FITC, Cyanine 3) and fluorescent proteins (BFP, CFP, GFP, YFP)
- Lyso-ID™ Red Detection Reagent specific signals (Excitation 568nm, Emission 667nm)

Lyso-ID™ Red Detection Kit can detect different forms of lysosome-dependent catabolic pathway dysregulation including phospholipidosis and autophagosome accumulation. Lyso-ID™ Red dye is suitable for monitoring cytotoxic lysosomal perturbation arising from drug or toxic agent treatment, with potential applications for in vitro ADME-Tox investigations by monitoring the aberrant intracellular accumulation of phospholipids induced by cationic amphiphilic drugs within live cells (phospholipidosis).

FIGURE 1: Composite bright-field and fluorescence microscopy images of control U2OS cells (left) and cells pre-treated with 64µM Chloroquine for 5 hours (right). Cells were stained with Lyso-ID™ Red dye for 10 minutes. Nuclei were counter-stained with Hoechst 33342 dye.

FIGURE 2: The Autophagy Pathway: The first step of autophagy consists of the progressive envelopment of cytoplasmic material in the phagophore. Next, the cytoplasmic material is sequestered in autophagosomes, which are delimited by two membranes. This two-membrane structure is a definitive characteristic of autophagic vacuoles. Autophagosomes then undergo a progressive maturation by fusion with endosomes or multi-lamellar bodies and/or lysosomes. This creates autolysosomes (also called autophagolysosomes) wherein the inner membrane and the luminal content of the autophagic vacuoles are degraded by lysosomal enzymes functioning within this acidic compartment. Lyso-ID™ Red dye is capable of detecting aberrant autophagosome accumulation induced by drugs such as Bafilomycin A.
Mito-ID™ Red Detection Kit
GFP-Certified™ for microscopy

Mito-ID™ Red Detection Kit contains a proprietary membrane-permeable mitochondria-selective dye that highlights mitochondria regardless of their energetic state. The kit is useful for assessing mitochondrial morphology changes, estimating mitochondrial mass and co-localizing GFP-tagged proteins to the mitochondrial compartment. Specifically designed for use with GFP-expressing cell lines, as well as cell lines expressing blue, cyan or yellow fluorescent proteins (BFPs, CFPs, YFPs).

FIGURE 1: Composite fluorescence microscopy images of HeLa cells (40x objective lens). Cells were stained with Mito-ID™ Red dye for 15 minutes. Nuclei were counter-stained with Hoechst 33342 dye.

Related Products

Mito-ID™ Red Antifade Reagents were developed specifically for Mito-ID™ Red mitochondrial stain when used for fixed cells and tissues. These reagents outperform other antifade reagents with little or no quenching. Use of Mito-ID™ Red Antifade Reagents with Mito-ID™ Red Detection Reagent significantly extends its photobleaching half-life. The addition of DAPI to the antifade reagent allows for staining of the nucleus.

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<td>Mito-ID™ Red Antifade Reagent</td>
<td>ENZ-53002</td>
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Mito-ID™ Membrane Potential Cytotoxicity Kit
homogeneous assay for microplates

- Ultrasensitive dual emission probe shifts from orange (590nm) to green (525nm) when mitochondrial membrane potential is compromised
- Easy to use and greater reproducibility due to higher photostability and better aqueous solubility
- True mix-and-read capability
- Mito-ID™ MP Detection Dye (Excitation 510nm, Emission 525nm, 590nm) dual emission

The Mito-ID™ Membrane Potential Kits measure mitochondrial membrane potential with a cationic dye that fluoresces either green or orange depending upon membrane potential status. In energized cells, the Mito-ID™ Membrane Potential reagent exists as a green-fluorescent monomer in the cytosol and also accumulates as orange-fluorescent aggregates in the mitochondria. However, in cells with compromised mitochondrial membrane potential, the Mito-ID™ Membrane Potential reagent exists primarily as green-fluorescent monomers throughout the cytosol and no longer exhibits orange fluorescence in the mitochondria. Compared with the commonly used cationic carbocyanine dye JC-1, Mito-ID™ Membrane Potential reagent offers greater solubility, better photostability, and higher sensitivity to mitochondrial membrane potential changes. A control mitochondrial membrane potential perturbation agent, carbonyl cyanide m-chlorophenylhydrazone (CCCP), is provided for monitoring changes in mitochondrial dynamics.

Mito-ID™ dye is highly sensitive to reductions in membrane potential. Figure 1 shows that Mito-ID™ dye detects membrane potential loss by CCCP at one tenth the concentration detected by the alternative dye JC-1. By enabling detection of very subtle mitochondrial responses to active compounds in a homogeneous assay format it is possible to screen unknown compounds for potential mitochondrial toxicity in secondary screening and ADME-Tox applications.

related product

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<td>Mito-ID™ Membrane Potential Detection Kit</td>
<td>ENZ-51018</td>
<td>100 Assays</td>
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Red Hydrogen Peroxide Assay Kit
for microplates

ENZ-51004 5 x 96 wells

KIT CONTAINS:
1 vial, CELLestial™ Red Peroxidase Substrate
20 units, Horseradish Peroxidase
200µl, H₂O₂ (Stock Solution)
1ml, DMSO
100ml, Assay Buffer

- Quantify hydrogen peroxide in solution, cell extracts or directly from certain live cells
- Detect a variety of oxidase activities through enzyme-coupled reactions
- Minimum of 10 picomoles of H₂O₂ detected
- Mix and read homogenous assay
- Compatible with HTS automation

Hydrogen peroxide (H₂O₂) is a reactive oxygen metabolic by-product that serves as a key regulator for a number of oxidative stress-related states. It is involved in a number of biological events and intracellular pathways that have been linked to several diseases. Measurements of reactive oxygen species help to determine how oxidative stress modulates varied intracellular pathways. The CELLestial™ Red Hydrogen Peroxide Assay Kit uses a non-fluorescent peroxidase substrate to quantify hydrogen peroxide in solution, in cell extracts and released from certain live cells (Figure 1). It can also be used to detect a variety of oxidase activities through enzyme-coupled reactions. The kit provides an optimized and homogenous assay format that is compatible with automated liquid handling systems. The CELLestial™ Red Hydrogen Peroxide Assay Kit offers a sensitive, one-step fluorometric assay to detect as little as 10 picomoles of H₂O₂ in a 100µl assay volume (Figure 2).

FIGURE 1: Conversion of Red Peroxidase Substrate into Resorufin: Horseradish peroxidase uses Red Peroxidase substrate as an electron donor during the reduction of hydrogen peroxide to water. The resultant product, resorufin, is a highly colored and fluorescent compound. (A) Use of assay for detection of glucose oxidase activity. (B) Use of assay in ELISA format to detect HRP-antibody conjugate.
Related Products

- ROS/RNS Detection Kit for microscopy

- Monitor reactive oxygen and/or nitrogen species in live cells
- Discriminates among superoxide, nitric oxide, and peroxynitrite
- Oxidative Stress Detection Reagent (Excitation 504nm, Emission 524nm)
- Superoxide Detection Reagent (Excitation 530nm, Emission 590nm)
- NO Detection Reagent (Excitation 648nm, Emission 666nm)

ROS/RNS Detection Kit enables simultaneous detection of different reactive species. Through the combination of three specific fluorescent probes, the kit provides a simple and specific assay for the real-time measurement of free nitric oxide (NO) and, by extension, nitric oxide synthase (NOS) activity, as well as global levels of reactive oxygen species (ROS), and specifically superoxide in living cells.

FIGURE 1: General profiling of reactive oxygen and nitrogen species formation by fluorescence microscopy was achieved in HeLa cells loaded with 3-Plex detection reagent from ROS/RNS Detection Kit and treated with pyocyanin (Panel A), or L-arginine (Panel B). General oxidative stress levels in the green channel, superoxide production in the orange channel, and NO in the Cy5/Red channel. Pretreatment with NAC, general ROS inhibitor, prevents formation of ROS (Panel A). cPTIO pretreatment abrogates NO synthesis (Panel B).

FIGURE 2: Flow chart for general profiling of ROS/RNS using 3-plex detection reagent and various inhibitors.

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Step 2</th>
<th>Step 3</th>
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<tr>
<td>Stain Cells</td>
<td>Excite Signal</td>
<td>Treat with Inhibitor and Determine Fluorescence</td>
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REACTIVE OXYGEN SPECIES / REACTIVE NITROGEN SPECIES

GREEN: NO Signal

ORANGE: NAC

RED: cPTIO

Fluorescent Signal - ROS/RNS Detection Protocol

Step 5a: Reactive oxygen detection confirmed

Step 5b: No signal is detected after treatment with inhibitors; check filter settings

www.enzolifesciences.com
Total ROS/Superoxide Detection Kit
for microscopy and flow cytometry

- **Monitor global levels of reactive oxygen species (ROS), and specifically superoxide in live cells**
- **Distinguish between hydrogen peroxide, peroxynitrite, and hydroxyl radicals**

The Total ROS/Superoxide Detection Kit provides real time reactive oxygen species (ROS) production in live cells. Two major components, the Oxidative Stress Detection Reagent (Green) and Superoxide Detection Reagent (Orange) combine to provide a simple and specific assay for real-time measurement of global levels of ROS and specifically superoxide in living cells.

**Related Products**

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<td>for fluorescence microscopy and flow cytometry</td>
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<td>for fluorescence microscopy</td>
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**FIGURE:** Profiling of reactive oxygen species formation by fluorescence microscopy was achieved in HeLa cells loaded with Enzo Life Sciences’ ROS/Superoxide detection reagents and treated with pyocyanin. General oxidative stress levels were monitored in the green channel, while superoxide production was detected in the orange channel. Pretreatment with NAC, a general ROS inhibitor, prevents formation of ROS.
New Screening and Optimization Strategies in GPCR-Based Drug Discovery

Drugs targeting members of the G protein-coupled receptor (GPCR) super-family represent the core of modern medicine [1]. With an estimated 600-1,000 members, the GPCR super family constitutes one of the largest families of molecular targets applicable to drug discovery in the human genome. They account for the majority of the best-selling drugs and roughly 40% of all prescription pharmaceuticals on the market today. Consequently, GPCRs are the target of choice for numerous high-throughput screening (HTS) campaigns designed to search for new drug leads of potential therapeutic significance [2].

Among fluorescence-based assays, measurement of transient calcium mobilization from intracellular stores in response to the activation of Gαq-coupled GPCRs is considered a standard approach to the pharmacological characterization of receptors and compounds and is frequently implemented in primary screening, as well as to generate a compound structure activity relation (SAR) for lead development programs. Ease of use and relatively low cost have been the primary motivations for the increased use of intracellular calcium assays in GPCR drug discovery. The basic principle of the cell-based assay is illustrated in Figure 1.

Activation of certain GPCRs does not lead to mobilization of intracellular calcium stores (Figure 2). However, most non-Gαq-coupled GPCRs can be adjusted to modulate intracellular calcium levels by co-transfection with promiscuous or chimeric/mutated G proteins making the calcium mobilization assay broadly applicable in GPCR research [2].
FluoForte™ Calcium Assay Kit

Enzo Life Sciences has introduced a new calcium-sensitive fluorescent dye, referred to as FluoForte™ reagent, which provides superior performance relative to the conventional dyes employed in such assays, i.e. Fluo-3 and Fluo-4. Our FluoForte™ Calcium Assay Kit, based upon the new fluorescent probe, is highly efficient, amenable to automation and provides a robust approach to screening GPCR agonists that mobilize calcium upon activation. Highlights of this kit include:

- **Homogeneous mix-and-read, no-wash calcium mobilization assay**
- **Load dye in live cells at 37°C or even room temperature for easier automation**
- **Broadly applicable to both GPCR and calcium ion channel targets**
- **Significantly brighter fluorescence intensity than competitors’ no-wash kits**
- **Provides comparable EC₅₀ values as obtained using Fluo-3, Fluo-4 and FLIPR® Calcium 4 assay kits**
- **No quencher dye, so no potential interference from compound ligand-receptor interactions**
- **Larger assay window than other dyes, allows measurements of challenging cell lines and receptors**
- **Stringently manufactured, to control and eliminate non-specific assay artifacts**

GFP-Certified™ FluoForte™ Calcium Assay Kit

Green fluorescent protein (GFP) is a benchmark standard of protein expression and intracellular localization in Biology. Unfortunately, the fluorescent properties of GFP complicate measurement of intracellular calcium mobilization using molecular probes, such as Fluo-3 and Fluo-4, which overlap spectrally with GFP. Fura-2 has been proposed as an alternative for monitoring calcium mobilization in GFP-expressing cells [3, 4]. However, Fura-2 is a UV-excitable dye not readily implemented on common high-throughput imaging plate readers, owing to the high background produced by polystyrene microplates and interference from fluorescent emission of test compounds at this excitation wavelength. Additionally, many microplate readers employ laser sources that limit use to probes excited at visible wavelengths. Rhod-2 is a red-emitting calcium probe, but is relatively dim and tends to concentrate in mitochondria. To address the need for measurement of calcium mobilization in GFP-expressing cells, a new red-emitting molecular probe was devised, providing superior performance compared to Fura-2 and Rhod-2. Our GFP-Certified™ FluoForte™ Calcium Assay Kit, based upon this new probe, is highly efficient, amenable to automation and serves as a robust approach to screening GPCR agonists that mobilize calcium upon activation. Highlights of this kit include:

- **Homogeneous mix-and-read, no-wash calcium mobilization assay**
- **The brightest and most sensitive red fluorescent Ca²⁺ indicator**
- **Designed and manufactured specifically for GFP-expressing cell lines**
- **Broadly applicable to both GPCR and calcium ion channel targets**
- **Provides comparable EC₅₀ values as obtained using Fluo-3, Fluo-4 and Rhod-2 AM**
- **No quencher dye, so no potential interference from compound ligand-receptor interactions**
- **Larger assay window than other dyes, allows measurement of compounds with weak signal response**
- **Stringently manufactured, to control and eliminate non-specific assay artifacts**

Literature References:

FluoForte™ Calcium Assay Kits
High-Throughput and Starter Pack for microplates

- Homogeneous mix-and-read, no-wash calcium mobilization assay
- Allows measurement of challenging cell lines and receptors
- Load dye in live cells at 37°C or room temperature for easier automation
- No quencher dye, so no potential interference from compound ligand-receptor interactions
- FluoForte™ Reagent (Excitation 490nm, Emission 514nm)

FluoForte™ Calcium Assay Kits are the brightest and most sensitive fluorescent Ca^{2+} indicators to detect calcium mobilization across a broad spectrum of biological targets. With a much higher signal intensity, FluoForte™ dye offers superior measurement of challenging cell lines and receptors. EC_{50} values (comparable to Fluo-3 and Fluo-4 dyes) and assay reproducibility have been benchmarked upon activation of a variety of G protein-coupled receptors (GPCRs). FluoForte™ is more than 2-fold brighter than Fluo-4, yields more robust signal (Z’-factor ≥ 0.75) and provides a larger assay window; making it suitable even when compounds elicit only a weak signal response.

**FIGURE 1:** Time course study of FluoForte™ reagent shows cell loading can be performed at room temperature or 37°C.

**FIGURE 2:** Cells were incubated with 100µl of 4µM Fluo-4 AM or FluoForte™ Reagent in HHBS at 37°C in a 5% CO_{2} cell culture incubator for 1 hour. The cells were washed twice with 200µl HHBS, ATP (20µl/well) was added to achieve a concentrations of 1µM with dye efflux inhibitor, then immediately imaged with a fluorescence microscope (Carl Zeiss, Inc.) using a FITC filter set.

**Related Products**

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FIGURE 1: Histamine dose response curves in Hela cells, expressing Histamine H1 receptors: Hela cells were seeded overnight at 40,000 cells per 100µl per well in a 96-well black wall/clear bottom microplate. The cells were incubated with 100µl of GFP-Certified™ FluoForte™ Reagent or Rhod-2 AM dye for 1 hour at 37°C. Histamine (20µl/well) was added using a BioTek two-syringe pump dispenser to achieve the final indicated concentrations. No significant difference in EC50 of Histamine for GFP-Certified™ FluoForte™ and Rhod-2 AM dye was observed. GFP-Certified™ FluoForte™ Reagent generated higher intensity signal and a larger assay window.

FIGURE 2: Cells were incubated with 100µl of 4µM Rhod-2 AM or GFP-Certified™ FluoForte™ Reagent in HHBS at 37°C in a 5% CO2 cell culture incubator for 1 hour. The cells were washed twice with 200µl HHBS, ATP (20µl/well) was added to achieve a concentrations of 1µM with dye efflux inhibitor, then immediately imaged with a fluorescence microscope (Carl Zeiss, Inc.) using rhodamine filter set.

GFP-Certified™ FluoForte™ Calcium Assay Kits
High-Throughput and Starter Pack for microplates

- Homogeneous mix-and-read, no-wash calcium mobilization assay
- Allows measurement of compounds with weak signal response
- Designed and manufactured specifically for GFP-expressing cell lines
- GFP-Certified™ FluoForte™ dye (Excitation 530nm, Emission 570nm)

Green fluorescent protein (GFP) is a benchmark standard of protein expression and intracellular localization in Biology. GFP-Certified™ FluoForte™ is a homogeneous calcium mobilization assay utilizing a new probe that is highly efficient and amenable to automation. The assay provides a robust approach to screening G protein-coupled receptor (GPCR) agonists that mobilize calcium upon activation. EC50 values (comparable to Fluo-3, Fluo-4 and Rhod-2 dyes) and assay reproducibility have been benchmarked upon activation of a variety of GPCRs and the assay has also been validated using GFP-expressing cell lines. The GFP-Certified™ FluoForte™ reagent is more than 4-fold brighter than Rhod-2 dye, yields more robust signal (Z'-factor ≥ 0.65) and provides a larger assay window; making it suitable even when compounds elicit only a weak signal response.

### Related Products

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<td></td>
<td>ENZ-52016-M001</td>
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GFP-Certified™ Apoptosis/Necrosis Detection Kit for microscopy and flow cytometry

- True multiplexing capabilities with GFP and other green fluorescent probes
- Distinguish between healthy, early apoptotic, late apoptotic and necrotic cells
- Perform death pathway analysis and drug/toxin studies
- Annexin V-cyanine 3 (Excitation 550nm, Emission 570nm) Red necrosis stain (Excitation 546nm, Emission 647nm)

Apoptosis/Necrosis Detection Kit (GFP-Certified™) readily distinguishes between early apoptosis, late apoptosis and necrosis, allowing detailed analysis of death pathways. The kit is specifically designed to minimize fluorescence signal overlap with probes that emit in the green region of the spectrum, such as GFP and FITC. Conjugation of Annexin V with an enhanced Cyanine 3 fluorophore enables simple detection of apoptosis upon binding to phosphatidylserine on the extracellular surface of suitably induced cells. Similarly, the Necrosis Detection Reagent (Red) detects loss of plasma membrane integrity by generating a far-red emission profile localized to the nucleus and distinct from both GFP and the Annexin V-cyanine 3 conjugate. Staurosporine is included as a positive control for induction of apoptosis. Applications ranging from target identification and validation to small molecule efficacy and toxicity enable both accurate and selective benchmarking of a cell state.

FIGURE 1: Excitation (hatched) and emission (solid) spectra for GFP, Annexin V-cyanine 3 conjugate and Necrosis Detection Reagent (Red). All three dyes are readily excited with a 488nm laser source. The emission maxima of all fluorophores are well separated from one another.

FIGURE 2: Apoptosis/necrosis induction in mitochondrial GFP-expressing HeLa cells. The Apoptosis Detection Reagent (Annexin V-cyanine 3) and Necrosis Detection Reagent (Red) specifically detect cell state with clear spectral separation from mitochondria-associated GFP signal. Healthy cells (A), cells undergoing apoptosis (B), cells undergoing late-stage apoptosis (C), and necrotic cells (D). Induction with 2µM Staurosporine was shown to be time-dependent, and yielded four distinct cell states after 4 hours.

KIT CONTENTS:
- 550µl, Apoptosis Detection Reagent (Annexin V)
- 600µl, Necrosis Detection Reagent (Red)
- 50nmoles, Apoptosis Inducer (Staurosporine)
- 6ml, 10X Binding Buffer

ENZ-51002-25    25 Assays
ENZ-51002-100  100 Assays

www.enzolifesciences.com
Nuclear-ID™ Green Chromatin Condensation Detection Kit for microscopy and flow cytometry

- Rapid analysis of apoptosis, based upon fluorescence detection of condensed chromatin in apoptotic cells
- Stains condensed chromatin of apoptotic cells ~40-fold more brightly than chromatin of healthy cells with control apoptosis-inducing agent, staurosporine
- Easy no wash, mix-and-read protocol
- Suitable for death pathway analysis and drug/toxin studies
- Nuclear-ID™ Green Detection Reagent (Excitation 503nm, Emission 531nm)

Nuclear-ID™ Green Chromatin Condensation Detection Kit provides a rapid and convenient assay for one of the more prominent hallmarks of apoptosis, nuclear condensation. The kit contains a DNA intercalating dye that brightly stains the condensed chromatin of apoptotic cells, but only dimly stains the normal chromatin of live cells. This staining pattern makes it possible to distinguish between healthy and apoptotic cell populations by fluorescence microscopy or flow cytometry. A control apoptosis-inducing agent, Staurosporine, is provided for monitoring apoptotic changes in nuclear organization. Potential applications for live-cell studies using the kit include monitoring the stages of chromatin condensation and rapid testing of compounds that induce apoptosis.

**FIGURE 1:** Chromatin condensation, as observed by fluorescence microscopy: (A) HeLa cells were treated for 4 hours with 0.2% DMSO (Control) or (B) 2µM Staurosporine on a slide and stained with 5µM Nuclear ID™ Chromatin Condensation dye. The dye brightly stains the condensed chromatin of apoptotic cells (arrow) while uncondensed chromatin of healthy cells are dimly stained.

**FIGURE 2:** Stages of Nuclear Chromatin Condensation during apoptosis.
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<th><strong>Violet (385-450nm)</strong></th>
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*Excitation and emission maxima in nanometers*
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- Cellular Analysis
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- Signal Transduction
- Cancer & Immunology
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Switzerland & Rest of Europe
ENZO LIFE SCIENCES AG
Industriestrasse 17, Postfach
CH-4415 Lausen / Switzerland
Tel. +41/0 61 926 89 89
Fax +41/0 61 926 89 79
info-ch@enzolifesciences.com

North/South America
ENZO LIFE SCIENCES INTERNATIONAL, INC.
5120 Butler Pike
Plymouth Meeting, PA 19462-1202 / USA
Tel. 1-800-942-0430/(610) 941-0430
Fax (610) 941-9252
info-usa@enzolifesciences.com

Benelux
ENZO LIFE SCIENCES BVBA
Melkerijweg 3
BE-2240 Zandhoven / Belgium
Tel. +32/0 3 466 04 20
Fax +32/0 3 466 04 29
info-be@enzolifesciences.com

Germany
ENZO LIFE SCIENCES GmbH
Marie-Curie-Strasse 8
DE-79539 Lörrach / Germany
Tel. +49/0 7621 5500 526
Toll Free 0800 6649518
Fax +49/0 7621 5500 527
info-de@enzolifesciences.com

UK & Ireland
ENZO LIFE SCIENCES (UK) LTD.
Palatine House
Matford Court
Exeter EX2 8NL / UK
Tel. 0845 601 1488 (UK customers)
Tel. +44/0 1392 825900 (overseas)
Fax +44/0 1392 825910
info-uk@enzolifesciences.com

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