Store at -20°C

#13837

Cell Health Assay Kit

www.cellsignal.com

1 Kit (1000 assays (96 well format))

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Orders: 877-616-CELL (2355)
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New 04/15

For Research Use Only. Not For Use In Diagnostic Procedures.

Description: The Cell Health Assay Kit is a fluorescence-based assay that determines cell viability by measuring intracellular esterase activity and plasma membrane integrity. The kit contains Calcein-AM, which stains viable cells, and Propidium Iodide (PI), an indicator of dead cells. Calcein-AM is the acetomethoxy form of calcein, a highly lipophilic, cell membrane permeable dye. Intracellular esterase converts the non-fluorescent Calcein-AM to the highly fluorescent calcein, which is retained within live cells and produces an intense green fluorescence. The DNA-binding agent Propidium Iodide is cell membrane impermeable and only enters dead cells or those with damaged cell membranes. Intracellular PI binds DNA and undergoes an approximate 40-fold enhancement in fluorescence intensity. As a result, live cells will produce a strong green fluorescence resulting from the conversion of Calcein-AM to calcein, while dead cells produce a strong red fluorescence due to the presence of Propidium Iodide.

At the recommended reagent concentrations and volumes, one kit will provide for 1000 assays (96-well plate format) or 200 flow cytometry assays.

Specificity/Sensitivity: The Cell Health Assay Kit will detect cell viability/toxicity in most eukaryotic cells. Between 500 and 50,000 cells/well can be used in this assay. A cell number titration is recommended when using a plate-reader with 96-well plate. For optimal conditions, titrations of Calcein-AM and Propidium Iodide are recommended for each cell line.

Background: Measures of cell viability and cytotoxicity are broadly used to study the effects of growth factors and cytokines, inhibitors and activators, and immune response signals. Many viability and toxicity assay kits (such as XTT, resazurin, and BrdU cell kits) monitor the presence or viability of live cells by measuring the metabolic conversion or incorporation of a substrate into cellular molecules. Assay kits that measure dead or dying cells usually rely on the activity of apoptotic enzymes (i.e. caspase-3) or the intracellular release of an enzyme or molecule indicative of cell death (i.e. cytochrome c, LDH). The Cell Health Assay Kit is able to stain both live and dead cells simultaneously by using two fluorescent probes. After treating cells with a combination of Calcein-AM and Propidium Iodide, live cells will produce strong green fluorescence while dead cells produce strong red fluorescence (1,2). These fluorescent signals can be detected using multiple methods, including a plate reader or scanner, flow cytometer, or fluorescent microscope. This dual assay is specifically designed to assess viability and toxicity for eukaryotic cells (1,2), but may also be used to assay some bacteria or yeasts (3,4).

Products Included	Product Number	Quantity
Calcein-AM	13844	550 µg x 2
Propidium Iodide (PI) Solution	11733	1.3 ml x 2

Note: This kit contains mixed storage components. Please store this entire kit at -20°C for long term storage. Upon first use, please allow components to thaw and then store each component as indicated on individual component labels.

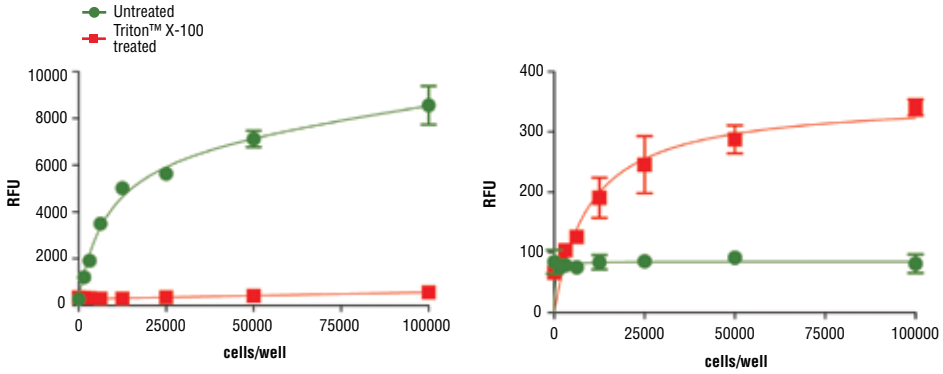
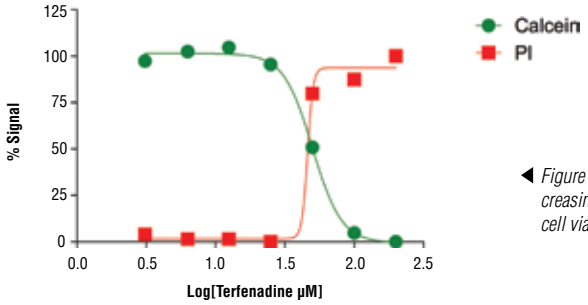


Figure 1. HeLa cells were seeded at 1,500 to 100,000 cells/well in a 96-well black plate with clear bottom and incubated overnight. Cells were exposed to Triton™ X-100 (0.05%, 10 min) and cell viability/toxicity was measured using the Cell Health Assay Kit #13837. Relative fluorescence for Calcein-AM (left) and Propidium Iodide (right) are shown.



◀ *Figure 2. Treatment of HeLa cells (4 x10⁴ cells/well) with increasing concentrations of terfenadine (4 hr) results in reduced cell viability as detected by the Cell Health Assay Kit.*

Background References:

(1) Papadopoulos, N.G. et al. (1994) *J Immunol Methods* 177, 101-11.

(2) Decherchi, P. et al. (1997) *J Neurosci Methods* 71, 205-13.

(3) Cárdenas, W. et al. (2004) *Fish Shellfish Immunol* 17, 223-33.

(4) Hiraoka, Y. and Kimbara, K. (2002) *Appl Environ Microbiol* 68, 2031-5.

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Cell Signaling TECHNOLOGY®

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

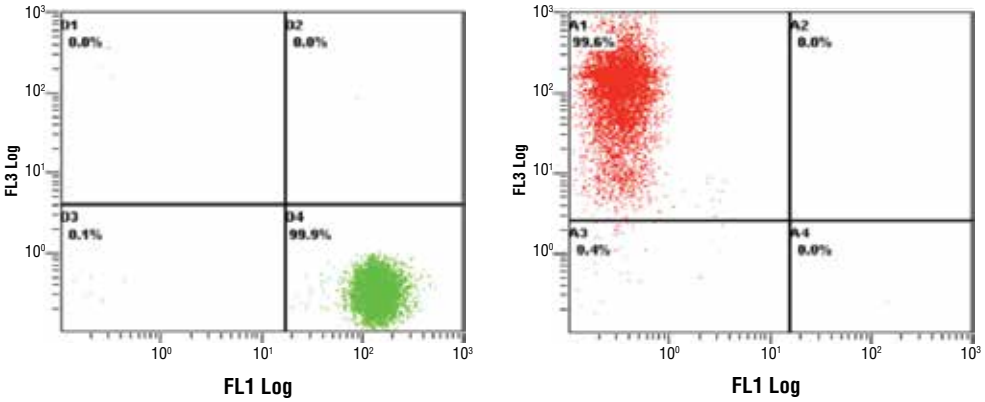

Figure 3. Flow cytometric analysis of Jurkat cells, untreated (green) or terfenadine-treated (50 µM, 4 hr; red), using Calcein-AM (0.1 µM, 20 min) and Propidium Iodide (3 µM, 20 min).

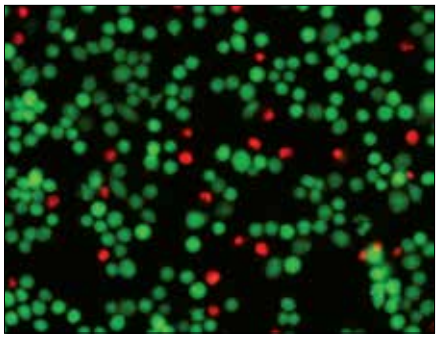

Figure 4. Fluorescent analysis of HeLa cells (4x10^4 cells/well) treated with terfenadine (12.5 µM, 4 hr) and stained with Calcein-AM (1 µM, 30 min; green) and Propidium Iodide (3 µM, 30 min; red).

Cell Health Assay Kit Protocol

A Solutions and Reagents

Kit Components

1. **Calcein-AM** (2X 550 µg, lyophilized)
2. **Propidium Iodide** (2X 150 µM, 1.3 ml)

Additional Reagents (Not Supplied)

1. **DMSO**
2. **20X Phosphate Buffered Saline (PBS) (#9808)**

B Instrumentation

1. Flow cytometer with excitation between 480 and 490 nm, and emission between 517 nm and 617 nm
2. Plate reader that can read 96-well plate with excitation about 490 nm and emission about 520 nm for calcein reading; excitation about 530 nm and emission about 617 nm for PI reading
3. 96-well black plate with clear bottom

C Reagent Preparation

Note: Allow all reagents to reach room temperature.

1. **1X PBS:** To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH2O, mix.
   Note: For flow cytometry application, adding 0.5% BSA to 1X PBS buffer may help to prevent cell loss.
2. **4 mM Calcein-AM:** Add 138 µl DMSO to each vial of Calcein-AM to make a 4 mM stock solution. Each vial contains enough 4 mM Calcein-AM stock solution for five 96-well plates or 100 flow cytometry assays. Calcein-AM solution should be used within 2 months after reconstitution and should be stored desiccated at -20°C and protected from light.
3. **Labeling Solution** (96-well plate assay): Prepare a 2 µM Calcein-AM, 3 µM PI in 1X PBS labeling solution by adding 5 µl of 4 mM Calcein-AM and 200 µl of 150 µM PI to 10 ml 1X PBS. This will provide enough labeling solution for one 96-well plate assay at recommended conditions.
   Note: For optimal labeling in different cell lines, a titration of both Calcien–AM and PI at final concentration between 0.1 to 10 µM is recommended for both live and dead cells. Treatment of cells with 0.05% Triton™ X-100 or 0.05% digitonin can be used to generate dead cells with damaged plasma membranes.

D Flow Cytometry Assay

1. Thaw reagents and bring to room temperature.
2. Harvest cells following desired treatments. Wash cells once with 1X PBS and create a 1 x 10^5 to 1 x 10^6 cell/ml cell suspension in 1X PBS. A 0.5 ml cell suspension (1 x 10^5-1 x 10^6 cells) per assay is recommended.
   Note: Adherent cells can be detached with EDTA. For toxicity assays, make sure to collect all dead cells floating in the medium.
3. Add Calcein–AM and PI to cell suspension. Recommended final concentration for Calcein-AM is 0.1-10 µM and the final PI concentration is 0.1-10 µM.
   Note: The final concentration of Calcein-AM for flow cytometry is much lower than that used for plate or microscopic applications. Dilute Calcein-AM in DMSO before each experiment. For example, dilute 1 µl of 4 mM stock Calcein-AM with 99 µl DMSO to make a 40 µM Calcein-AM working solution. This must be used within one day. Add 1.25 µl of 40 µM Calcein-AM working solution and 20 µl of 150 µM PI to each 1 ml cell suspension to reach a final concentration of 0.05 µM Calcein-AM and 3 µM PI.
4. Mix well and incubate cells at room temperature for 15 to 30 min. Protect from light.
5. Analyze samples on a flow cytometer set at excitation/emission of 488/535 nm for live cells, and an excitation/emission setting of 488/620 nm for dead cells.

E 96-well plate Assay

For cells in suspension:

1. Harvest cells and wash once with 1X PBS. Make a 1 x 10^5 to 1 x 10^6 cell/ml cell suspension with 1X PBS. Proceed to step 3.

For adherent cells:

2. Seed cells into 96-well plate in warm culture medium and culture cells in incubator overnight to allow cells to attach to plate. Typical cell number is between 1 x 10^4 to 5 x 10^4 cells/ well.
   Note: A cell number titration may be necessary for optimal results (see Figure 1).
3. Remove the medium from the plate and wash cells once with 1X PBS. Add 100 µl/well of 1X PBS to plate followed by treatment with desired growth factors or cytotoxic reagents.
   Note: Because labeling solution will be added directly to this plate, use an FBS-free cell treatment to avoid calcein signal loss due to serum esterase.
4. Add 100 µl/well of labeling solution to cell plate and incubate cells at room temperature for 30 to 60 min while protected from light.
5. Analyze samples on a plate reader or fluorescent microscope set at excitation/emission of 490/520 nm for live cells, and an excitation/emission setting of 535/620 nm for dead cells.