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Product Information

Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells

Catalog Number: 30002-T, 30002

Kit Contents

Component	30002-T, 150 assays*	30002, 300 assays*
30002A Calcein AM 4 mM in anhydrous DMSO	1 vial (50 uL)	2 vials (50 uL each)
99905: EthDIII 2 mM in DMSO/H_2O	1 vial (150 uL)	2 vials (150 uL each)

* Assay number is based on 0.5 mL staining volume at 2 uM calcein AM/4 uM EthD-III. Number of assays may vary depending on the staining volume and optimal dye concentrations for your application.

Storage and Handling

Store kit at -20°C, desiccated and protected from light. Product is stable for at least 6 months from date of receipt when stored as recommended. Note: aqueous solutions of calcein AM are susceptible to hydrolysis. Working solutions of calcein AM diluted in buffer should be used within one day of preparation. Working solutions of EthD-III in buffer can be stored at -20°C, protected from light, for at least one year.

Spectral Properties

Calcein (end product after hydrolysis of calcein AM):
Ex/Em: 494/517 nm (pH 8)

Ethidium homodimer III (EthD-III):
Ex/Em: 522/593 nm* (with DNA)
*Ethidium Homodimer III also has a strong UV absorbance peak at 279 nm

Product Description

The Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells provides green/red fluorescent staining of viable and dead cells, respectively, using probes for intracellular esterase activity in viable cells, and compromised plasma membrane integrity in dead cells (1,2). The assay is a sensitive and non-toxic alternative to ^{51}Cr release, trypan blue exclusion, and similar assays.

Calcein AM is a membrane-permeable, non-fluorescent esterase substrate, which enters the cytoplasm and is cleaved by esterases in live cells to yield the green fluorescent dye calcein. Calcein is negatively charged and cell membrane-impermeable, and consequently is retained in the cytoplasm of viable cells with intact plasma membranes. Dead cells either do not stain with calcein due to lack of esterase activity, or fail to retain calcein in the cytoplasm due to compromised plasma membrane integrity.

Ethidium homodimer III (EthD-III) is a plasma membrane-impermeable DNA dye that is excluded by viable cells. EthD-III is virtually non-fluorescent until it binds DNA, upon which it undergoes a 25-fold enhancement of fluorescence. EthD-III penetrates dead cells with compromised plasma membranes and stains the nucleus with bright red fluorescence. EthD-III was developed at Biotium, and is spectrally similar to Ethidium homodimer I (EthD-I), but stains DNA with 45% brighter fluorescence.

Note that calcein AM-based assays can be used in adherent or suspension cultures of eukaryotic cells (3) and certain tissues (4), but cannot be used in yeast or bacteria. See the related products table (next page) for Biotium's bacterial viability/cytotoxicity assays.

Assay Protocols

Note: This assay must be used on unfixed cells. The dyes cannot be used for live/dead discrimination in fixed cells or tissues, and cannot withstand fixation after staining.

For fluorescence microscopy

1. Warm the dye stock solutions to room temperature. Prepare a staining solution of 2 uM calcein AM/4 uM EthD-III by adding 5 uL of 4 mM calcein AM and 20 uL of 2 mM EthD-III to 10 mL of PBS or other serum-free buffer or medium. Vortex to ensure thorough mixing.

   Note: Volumes may be scaled proportionally as needed.

   Note: Optimal dye concentrations may vary depending on cell type. In general it is best to use the lowest dye concentration that gives sufficient signal. Typical staining concentrations range between 0.1 uM and 10 uM for Calcein AM and EthD-III.

   Note: Aqueous solutions of calcein AM are susceptible to hydrolysis. Working solutions of calcein AM diluted in buffer should be used within one day of preparation. Working solutions of EthD-III can be stored at -20°C, protected from light, for at least one year.

2. Wash the cells twice with serum-free buffer or medium to remove serum esterase activity. For suspension cells, pellet cells by centrifugation, remove the supernatant, and resuspend in wash buffer; repeat once.

   Note: Dead cell controls can be prepared by treating cells with 0.1% saponin or 0.1-0.5% digitonin for 10 minutes.

3. For adherent cells, add a sufficient volume of calcein AM/EthD-III staining solution to cover the cell monolayer. For suspension cells, resuspend the washed cell pellet in staining solution at or below the typical cell density of a confluent culture.

4. Incubate the cells for 30-45 minutes at room temperature.

5. Image the labeled cells by fluorescence microscopy. Calcein and EthD-III can be viewed simultaneously with a conventional fluorescein long pass filter, or the dyes can be imaged separately; calcein can be viewed with a standard fluorescein bandpass filter and EthD-III can be viewed with filters for rhodamine, propidium iodide or Texas Red®.

   Optional: The staining solution can be removed and replaced with fresh buffer or medium or your choice prior to imaging. For suspension cells, pellet the cells by centrifugation, remove the staining solution, and resuspend the cells in fresh buffer or medium.

For flow cytometry

1. Stain cells in suspension (or trypsinized adherent cells in suspension) according to the protocol for fluorescence microscopy.

2. Pellet the cells by centrifugation and resuspend in your preferred buffer for flow cytometry analysis.

3. Analyze calcein fluorescence in the fluorescein channel, and EthD-III fluorescence in the channel for either propidium iodide or Texas Red®.

For fluorescence microplate reader

1. Grow adherent cells or aliquot suspension cells in well of a 96-well microplate.

   Note: The range of detection for cells is usually between 200-500 and 10^6 cells per well of a 96-well plate.

   Note: Dead cells can be obtained for use as a control by treatment with 0.1% saponin or 0.1-0.5% digitonin for about 10 minutes.

2. Prepare a staining solution of 2 uM calcein AM/4 uM EthD-III. Warm the dye stock solutions to room temperature. Add 20 uL of 2 mM EthD-III and 5 uL of 4 mM calcein AM to 10 mL of PBS or other serum-free buffer or medium. Vortex to mix well.

   Note: The 10 mL of staining solution is sufficient for one 96-well microplate; volumes may be scaled proportionally as needed.

Note: Optimal dye concentrations may vary depending on cell type. In general it is best to use the lowest dye concentration that gives sufficient signal. Typical staining concentrations range between 0.1 uM and 10 uM for calcein AM and EthD-III.

Note: Aqueous solutions of calcein AM are susceptible to hydrolysis. Working solutions of calcein AM diluted in buffer should be used within one day of preparation. Working solutions of EthD-III can be stored at -20°C, protected from light, for at least one year.

3. Wash the cells in serum-free buffer or medium to remove serum esterase activity. For adherent cells in a 96-well plate, wash with 100 uL buffer per well. For suspension cells, pellet the cells by centrifugation in the plate and then resuspend the cells in 100 uL serum-free medium or buffer; repeat once.
4. Add 100 uL serum-free buffer to each well. For suspension cells, resuspend in 100 uL serum-free buffer per well.
5. Add 100 uL of the Calcein AM/EthD-III working solution to each well. This results in a final volume of 200 uL per well, and final concentrations of 1 uM calcein AM and 2 uM EthD-III. Pipet gently up and down, or shake the plate on an orbital shaker to mix well.
6. Incubate the samples at room temperature for 30–45 minutes.
7. Measure fluorescence using a microplate reader. Calcein can be detected using settings for fluorescein, while EthD-III can be detected using settings for rhodamine or Texas Red®. See spectral properties for optimal excitation/emission wavelengths.

   Note: Relative fluorescence values (RFU) can be compared between samples to measure changes in the number of live or dead cells in a sample relative to a reference sample. See below for alternative methods of data analysis.

Determining the percentage of live and dead cells in a population

The following controls can be used to determine the percentage of live or dead cells in a population. These include dead cell controls, healthy cell controls, and cell-free controls. Dead cell controls can be prepared by treating cells with 0.1% saponin or 0.1-0.5% digitonin for about 10 minutes.

1. Prepare working solution and stain cells as described in the microplate protocol above. In addition, prepare 1 mL each of separate solutions of 2 uM calcein AM alone and 4 uM EthD-III alone to stain the controls as indicated below.
2. Measure fluorescence of experimental and control samples:

   A. Fluorescence at 645 nm in the experimental cell sample, labeled with calcein AM and EthD-1 = $F(645)_{sam}$

   B. Fluorescence at 530 nm in the experimental cell sample, labeled with calcein AM and EthD-1 = $F(530)_{sam}$

   C. Fluorescence at 645 nm in a sample where all the cells are dead, labeled with EthD-1 only = $F(645)_{max}$

   D. Fluorescence at 645 nm in a sample where all the cells are dead, labeled with calcein AM only = $F(645)_{min}$

   E. Fluorescence at 530 nm in a sample where the majority of cells are alive, labeled with EthD-1 only = $F(530)_{min}$

   F. Fluorescence at 530 nm in a sample where the majority of cells are alive, labeled with calcein AM only = $F(530)_{max}$

   G. Fluorescence at 530 nm of the cell-free sample with or without dye added = $F(530)_0$

   H. Fluorescence at 645 nm of a cell-free sample with or without dye added = $F(645)_0$
3. Calculate the percentages of live and dead cells from the fluorescence readings:

   % Live Cells = (B - E) ÷ (F - E)

   % Dead Cells = (A - D) ÷ (C - D)

Determining absolute numbers of live and dead cells in a population

The absolute number of live and dead cells in a sample can be obtained by constructing a standard curve of cell number versus fluorescence at 530 nm and at 645 nm. The fluorescence intensity of each dye is linearly related to the total number of live or dead cells present in the sample, respectively.

References

1. Principles and Methods of Toxicology, Third Edition, A.W. Hayes, Ed., Raven Press (1994) pp. 1231–1258.
2. Papadopoulos NG, et al. J Immunol Methods 177, 101 (1994).
3. Vaughan PJ, et al. J Neurosci 15, 5389 (1995).
4. Poole CA, et al. J Cell Sci 106, 685 (1993).

Related Products

Catalog number	Product
30026	Calcein AM Cell Viability Assay Kit
30025	Resazurin Cell Viability Assay Kit
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay Kit
30068	ViaFluor® 405 SE Cell Proliferation Kit
30086	ViaFluor® 488 SE Cell Proliferation Kit
30050	ViaFluor® CFSE SE Cell Proliferation Kit
10407	NucView® 405 Caspase-3 Substrate
10403	NucView® 488 Caspase-3 Substrate
10408	NucView® 530 Caspase-3 Substrate
32002-32009	Live-or-Dye™ Fixable Viability Staining Kits (choose from 8 fluorescent dye colors)
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30067	Dual Apoptosis Assay with NucView® 488 Caspase-3 Substrate and CF®594 Annexin V
70070	MitoView™ 405
70054	MitoView™ Green
70055	MitoView™ 633
70075	MitoView™ 650
70068	MitoView™ 720
30001	JC-1 Mitochondrial Membrane Potential Detection Kit
30019	MCB Glutathione Detection Kit
30027	Viability/Cytotoxicity Assay Kit for Bacteria Live & Dead Cells
32001	Bacterial Viability and Gram Stain Kit
31033-31037	PMA Real-Time PCR Bacterial Viability Kits (choose from kits for 5 bacterial strains)
40069	PMAxx™, 20 mM in Water for viability PCR
40019	PMA Dye, 20 mM in Water for viability PCR

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