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Product Information

Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells

Catalog Numbers: 30002, 30002-T

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: EthD-III 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 and concentrations of 2 uM calcein AM and 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. Working solutions of calcein AM diluted in buffer should be used within one day of preparation. Working solutions of EthD-III diluted in buffer can be stored at -20°C, protected from light, for at least one year.

Spectral Properties

Component	Ex/Em maxima	Detection channel for flow	Detection channel for microscopy
Calcein (end product)	494/517 nm	FITC	FITC
EthD-III	532/625 nm* (with DNA)	PE or PE-TexasRed®	TexasRed® or Cy®3

*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 a convenient assay for detecting viable cells (green) and dead cells (red) within the same cell population by flow cytometry, fluorescence microscopy, or plate reader. The assay is a sensitive and non-toxic alternative to ^{51}Cr release, trypan blue exclusion, and similar assays.

Calcein AM is a membrane-permeant, 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-impermeant, 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-impermeant 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, spheroid or Matrigel® 3D-cell culture models (1), and certain live tissue preparations (2), but cannot be used in yeast or bacteria.

General Assay Considerations

1. 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.
2. 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. 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.
4. Dead cell controls can be prepared by treating cells with 0.1% saponin or 0.1-0.5% digitonin for 10 minutes. Alternatively, cells can be killed by heating to 56°C for 45 minutes, and then cooled to room temperature.
5. To determine the absolute number of live and dead cells in a sample using the plate reader assay, prepare standard curves of known numbers of healthy cells and dead cells. Plot the fluorescence at 517 nm and at 625 nm versus cell number, and for samples.

Assay Protocols

For fluorescence microscopy

1. Warm the dye stock solutions to room temperature and vortex to mix.
2. Prepare a staining solution of 2 uM calcein AM and 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.
3. 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.
4. For adherent cells, add a sufficient volume of the 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.
5. Incubate the cells for 30-45 minutes at room temperature.
6. Optional: The staining solution can be removed and replaced with fresh buffer or medium of 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.
7. Image the labeled cells by fluorescence microscopy. Calcein can be viewed imaged using a FITC filter set, and EthD-III can be imaged using TexasRed® or Cy®3 filter sets.

For flow cytometry

1. Stain cells in suspension according to the protocol for fluorescence microscopy (steps 1-5).
2. Pellet the cells by centrifugation and resuspend in your preferred buffer for flow cytometry analysis.
3. Perform flow cytometry analysis, detecting calein in the FITC channel, and EthD-III in the PE or PE-TexasRed® channel.

For fluorescence microplate reader

1. Grow adherent cells or aliquot suspension cells in wells 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.
2. Warm the dye stock solutions to room temperature and vortex to mix.

3. Prepare a staining solution of 2 uM calcein AM and 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: 10 mL of staining solution is sufficient for one 96-well microplate; volumes may be scaled proportionally as needed.

4. 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.

5. Add 100 uL serum-free buffer to each well. For suspension cells, resuspend in 100 uL serum-free buffer per well.

6. 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.

7. Incubate the samples at room temperature for 30–45 minutes.

8. Measure fluorescence using a microplate reader. Calcein can be detected using settings for fluorescein, with peak emission at 517 nm. EthD-III can be detected using settings for rhodamine or Texas Red®, with peak emission at 625 nm.

   Note: See General Assay Considerations on the previous page for instructions on using a standard curve to estimate absolute numbers of live or dead cells.

References

1. Biotechniques (2008) 45:165-171. doi:10.2144/000112883
2. J Orthop Res (2013) Oct;31(10):1514-9. doi: 10.1002/jor.22405

Related Products

Catalog number	Product
30065	Apoptosis and Necrosis Quantitation Kit Plus
99902	5X Annexin V Binding Buffer
29003-29085	Annexin V CF® Dye Conjugates
29006-29082	Annexin V Near IR CF® Dye Conjugates
29003R-29085R	Annexin V CF® Dye Conjugates, Azide-Free, Lyophilized
30072	NucView® 488 and RedDot™2 Apoptosis and Necrosis Kit
10402	NucView® 488 Caspase-3 Substrate, 1 mM in DMSO
10405	NucView® 405 Caspase-3 Substrate, 1 mM in DMSO
10406	NucView® 530 Caspase-3 Substrate, 1 mM in DMSO
30062	NucView® 488 and MitoView™ 633 Apoptosis Kit
30063	CF®488A TUNEL Assay Apoptosis Detection Kit
30064	CF®594 TUNEL Assay Apoptosis Detection Kit
70055	MitoView™ 633
32002-32013	Live-or-Dye™ Fixable Viability Staining Kits
32010	Live-or-Dye™ NucFix Red Staining Kit
30020	ATP-Glo™ Bioluminometric Cell Viability Assay
30068	ViaFluor® SE 405 Cell Proliferation Kit
30086	ViaFluor® SE 488 Cell Proliferation Kit
30050	ViaFluor® SE CFSE Cell Proliferation Kit

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