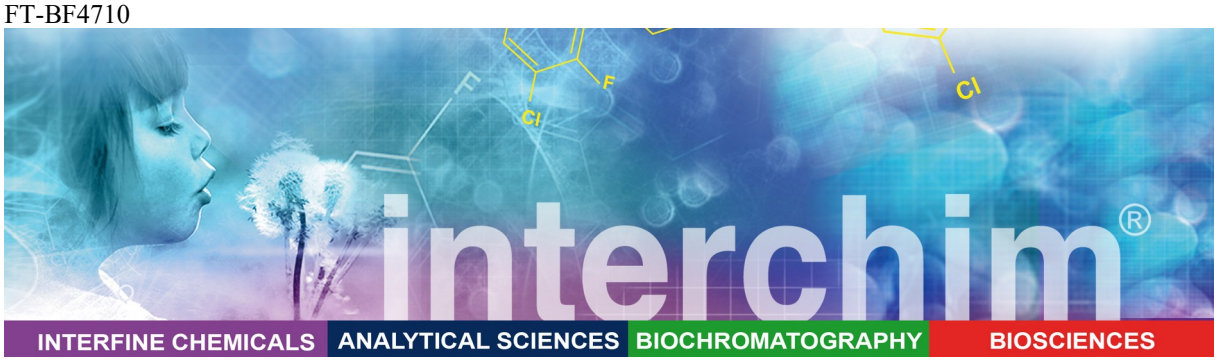


Live/Dead MammalianViability/Cytotoxicity Assay Kit

Product Information

Name : **Vability / Cytotoxicity Assay kit for Live & Dead cells**

Catalog Numbers : FP-BF4710 ~300 tests in flow cytometer
~1000 tests in fluorescence microscope / fluorescence microplate reader

Product Components :

	Quantity	Composition	Spectral Properties
Calcein AM	2*50 µl	4 mM in DMSO	494/517 nm (pH 8)
EthD-III	2*150 µl	2 mM in DMSO/H2O 1:4 (v/v)	522/593 nm (with DNA) also absorbance at 279 nm

Storage: Reagents in this kit should be stored sealed, desiccated, protected from light and frozen at -20°C. Allow the reagents to warm to room temperature for 30 min and centrifuge briefly before opening. Before refreezing, seal all stock solutions tightly. Calcein AM is susceptible to hydrolysis when exposed to moisture. If the color of Calcein AM stock solution turns orange, discard the tube. Prepare aqueous working solutions containing Calcein AM immediately prior to use, and use within 8 hours. EthD-III is stable and insensitive to moisture. Stock solutions of EthD-III in DMSO/ H2O or other aqueous media can be stored frozen at -20°C for at least one year.

Introduction

The Viability/Cytotoxicity Assay Kit for Live & Dead Cells provides a two-color fluorescence staining on both live and dead cells using two probes that measure two recognized parameters of cell viability — intracellular esterase activity and plasma membrane integrity [Hayes 1994, Papadopoulos 1994)]. The kit is suitable for use with fluorescence microscopes, fluorescence multiwell plate scanners and flow cytometers and other fluorescence detection systems. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells [Vaughan 1995] and certain tissues [Poole 1993], but not to bacteria or yeast. This fluorescence-based method of assessing cell viability can be used in place of trypan blue exclusion, 51Cr release and similar methods for determining cell viability and cytotoxicity. It is generally faster, less expensive, safer and a more sensitive indicator of cytotoxic events than alternative methods. EthD-III shares the same property with EthD-I used in Live/Dead Viability/Cytotoxicity Assay Kit and is 40% brighter at intensity compared to EthD-I. Validity of the Live/Dead Viability/Cytotoxicity assay for animal cell applications has been established by several publications.
Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein. The polyanionic dye calcein is well retained within live cells, producing an intense uniform green fluorescence in live cells (ex/em

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

~495nm/~515 nm). EthD-III enters cells with damaged membranes and undergoes a 25-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-III is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually nonfluorescent before interacting with cells.

Directions for use

Protocol 1 - 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.

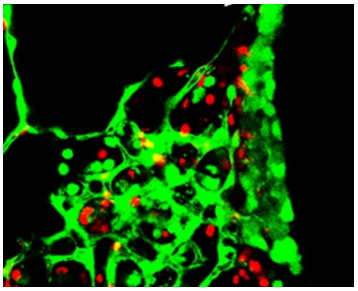
2- Wash the cells twice with serum-free buffer or medium to remove serum esterase activity. For suspension cells, pellet cells by centrifugation, remove the supernatant, and resuspend in wash buffer; repeat once.

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.

Longpass and dual emission filters useful for simultaneous viewing of calcein and EthD-III stains
Omega Filters: XF25, XF26, XF115
Chroma Filters: 11001, 41012, 71010
Bandpass filters for viewing calcein alone
Omega Filters: XF22, XF23
Chroma Filters: 31001, 41001
Bandpass filters for viewing EthD-III alone
Omega Filters: XF32, XF43, XF102, XF108
Chroma Filters: 31002, 31004, 41002, 41004



(Goris, 2009)

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

Protocol 2 - 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®.

Protocol 3 - 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 200uL 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.

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

FT-BF4710

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:

$$\% \text{ Live Cells} = \frac{B - E}{F - E}$$

$$\% \text{ Dead Cells} = \frac{A - D}{C - D}$$

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.

Related products

- Live/Dead Bacterial Viability/Cytotoxicity Assay Kit, FP-BU1040
- Calcein AM, FP-895515
- EthD-III, BP9340
- DMAO, green nucleus marker for live & dead cells, CA8150
- Propidium monoazide (PMA), FP-BZ9340

References

- "Principles and Methods of Toxicology", *Third Edition, A.W. Hayes, Ed., Raven Press* pp. 1231, (1994)
- **Cubells JF**, *et al.*, "Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress.", *J Neurosci*, **14**, 2260 (1994)

LIFE SCIENCES
interchim®
211bis Avenue J.F. Kennedy - BP 1140 - 03103 Montluçon Cedex - France
S.A. a conseil de surveillance 1.548.000 euro - RCS Montluçon 917 050 171 -APE 4669B

- **Forte M**. *et al*., Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis, *PNAS*, 104, no. 18:7558-7563 (2007) Article
- **Goris K**. *et al*., Differential Sensitivity of Differentiated Epithelial Cells to Respiratory Viruses Reveals Different Viral Strategies of Host Infection, *Journal of Virology*, p. 1962-1968, Vol. 83, No. 4 (2009) Article
- **Hauser JM**, *et al.*, "Role of ceramide in mitogenesis induced by exogenous sphingoid bases.", *J Biol Chem*, **269**, 6803 (1994).
- **Jacobsen MD**, *et al.*, "Role of Ced-3/ICE-family proteases in staurosporine-induced programmed cell death.*", J Cell Biol*, **133**, 1041 (1996).
- **Kirshenbaum LA**, *et al.*, "Adenovirus E1A Represses Cardiac Gene Transcription and Reactivates DNA Synthesis in Ventricular Myocytes, via Alternative Pocket Protein- and p300-binding Domains.", *J Biol Chem*, **270**, 7791 (1995).
- **Mulhern M**. *et al*., The Unfolded Protein Response in Lens Epithelial Cells from Galactosemic Rat Lenses, *Investigative Ophthalmology and Visual Science*. 47:3951-3959 (2006) Article
- **Papadopoulos NG**, *et al.*, "An improved fluorescence assay for the determination of lymphocyte-mediated cytotoxicity using flow cytometry", *J Immunol Methods*, **177**, 101 (1994).
- **Pike CJ**, *et al.*, "Amino-terminal Deletions Enhance Aggregation of -Amyloid Peptides in Vitro.", *J Biol Chem*, **270**, 23895 (1995).
- **Poole CA**, *Brookes NH, Clover GM.*, "Keratocyte networks visualised in the living cornea using vital dyes.", *J Cell Sci*, **106**, 685 (1993).
- **Sontheimer H**, *et al.*, "Astrocyte Na+ channels are required for maintenance of Na+/K(+)-ATPase activity.", *J Neurosci*, **14**, 2464 (1994).
- **Vaughan PJ**, *et al.*, "Thrombin receptor activation protects neurons and astrocytes from cell death produced by environmental insults.", *J Neurosci*, **15**, 5389 (1995).
- **Wang XM**, , *et al.*, "Anew microcellular cytotoxicity test based on calcein AM release.", *Hum Immunol*, **37**, 264 (1993).
- **Weil M**, *et al.*, "Constitutive expression of the machinery for programmed cell death.", *J Cell Biol* ,**133**, 1053 (1996).
- **Yang W.** *et al*., Mutations during the Adaptation of H9N2 Avian Influenza Virus to the Respiratory Epithelium of Pigs Enhance Sialic Acid Binding Activity and Virulence in Mice, J. Virol. vol. 91 no. 8 (2017) Article

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06