CREATIVE BIOMART®
Assay Kit

Live/Dead Assay Kit

Product Information

Common Name

Cell

Cat.No. Kit-2168

Product Overview

The Live/Dead Assay Kit provides a two-color fluorescence assay that detects both live and dead cells for mammalian cell types.

Description

This kit enables the detection of both the live and dead populations of mammalian cells based on the integrity of the cell membrane using a fluorescence microplate reader. Calcein AM is a widely used live-cell marker. The non-fluorescent calcein AM permeates the intact cell membrane and is converted into the fluorescent calcein by intracellular esterases. The number of live cells is therefore indicated by the intensity of green fluorescence in the cytosol, with excitation at 495 nm and emission at 530 nm. EthD-III is virtually non-fluorescent and impermeant to an intact plasma membrane. In the event of compromised cell membrane integrity that is associated with cell death, EthD-III enters cells and binds to nucleic acids, resulting in bright red fluorescence in dead cells, with excitation at 530 nm and emission at 645 nm.

Stability

When stored as directed, the kit is stable for at least 6 months from the date it is received.

Storage

Reagents in this kit should be stored sealed, desiccated, protected from light, and frozen at -20°C. Allow the reagents to warm up to room temperature 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. 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.

Synonyms

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Fax:1-631-938-8127
45-1 Ramsey Road, Shirley, NY 11967, USA

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Cell Assay Kit

Size

200 Tests

Kit Components

Calcein AM (4 mM, 30 µL)
EthD-III (2 mM, 60 µL)
Each kit contains enough material for two (2) 96-well or 384-well microplates.

Materials Required but Not Supplied

Black-walled, clear bottomed microplates (96-well);
Phosphate-Buffered Saline (PBS)

Features & Benefits

Optimized for fluorescence microplate readers, such as SpectraMax® readers
Simple workflow-direct measurement in wells with or without medium removal
Increased sample throughput with microplate format
Preconfigured protocol in SoftMax® Pro Software

Preparation

Use the following procedure to prepare 6 µM calcein AM and 6 µM EtD-III:
1. Remove the calcein AM and EthD-III reagent stock solutions from the freezer and allow them to warm to room temperature.
2. Add 30 µL of the 2 mM EthD-III and 15 µL of 4 mM calcein AM to 10 mL of PBS, and then vortex to ensure thorough mixing.
Note: 10 mL of this working solution is sufficient for one 96-well microplate.
3. Optionally, if the percentage of live or dead cells in the population needs to be determined, for live-only and dead-only controls, prepare 1 mL of 6 µM calcein AM only solution and 1 mL of 6 µM EthD-III only solution.
CAUTION: Because aqueous solutions of calcein AM are susceptible to hydrolysis, use aqueous working solutions within one day.

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Assay Protocol

1. Plate 20000 cells in 100 µL culture medium per well in a black-walled, clearbottomed, 96-well microplate. Incubate in a 37°C, 5% CO2 incubator overnight. If needed, include wells without cells as a background control.
Molecular Devices recommends seeding cells at sufficient density to form a confluent.
For suspension cells, the recommended cell density is 40000 to 200000 cells per well.
For adherent cells, the recommended cell density is 20000 to 80000 cells per well.
2. Prepare samples of live cells and dead cells for control. Typically, dead cell controls can be prepared by treatment of live cells with 0.1% saponin or 0.1 to 0.5% digitonin, or another method of your choosing, for about 10 minutes.
3. Treat cells using the methods of your choosing for cell viability assays. Remember to include untreated wells as controls.
4. Add 100 µL of the calcein AM/EthD-III working solution to each well, which results in a final volume of 200 µL per well and a final concentration of 3 µM calcein AM and 3 µM EthD-III.
5. Incubate the samples at room temperature for 30 to 60 minutes, protected from light.
6. Measure fluorescence in a fluorescence microplate reader with the following setup:
For live cells: with excitation at 495 nm and emission at 530 nm.
For dead cells: with excitation at 530 nm and emission at 645 nm.
Fluorescence Microplate Reader Setup with SoftMax Pro Software
Read Mode: Fluorescence
Read Type: Endpoint
Wavelengths: Lm1: Ex = 495 nm, Em = 530 nm; Lm2: Ex = 530 nm, Em = 645 nm
PMT and Optics: PMT Gain: Automatic; Flashes perread: 6; Read From Bottom*
*Bottom read is preferred for cell-based assays, but top read can be used with microplate readersthat do not have the bottom read option.
Determining the Percentage of Live and Dead Cells in a Population:
Sample Label Added
Experimental cells: Calcein AM and EthD-III
All (or mostly) live cells: EthD-III only
All (or mostly) live cells: Calcein AM only

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All dead cells: EthD-III only
All dead cells: Calcein AM only
Cell-free control (optional): Dye optional*
*You can set up the cell-free control with or without dye added.
Measure the fluorescence in the experimental cell samples and the control cell samples using both of the following wavelength pairs:
Excitation at 495 nm and emission at 530 nm
Excitation at 530 nm and emission at 645 nm

Analysis

Calculate the percentage of live cells from the fluorescence readings at the emission wavelength 530 nm according to the following formula:
% Live Cells = (A - B)/(C - B) x 100
Where:
A. A is fluorescence at the emission wavelength 530 nm in the experimental cell sample, labeled with calcein AM and EthD-III.
B. B is fluorescence at the emission wavelength 530 nm in a sample where most of the cells are alive, labeled with EthD-III only.
C. C is fluorescence at the emission wavelength 530 nm in a sample where most of the cells are alive, labeled with calcein AM only.
Optionally, to subtract background fluorescence, measure the fluorescence of the cellfree control at the emission wavelength 530 nm. Then, subtract the background fluorescence measured at the emission wavelength 530 nm from values A, B, and C.
Calculate the percentage of dead cells from the fluorescence readings at the emission wavelength 645 nm according to the following formula:
% Dead Cells = (D - E)/(F - E) x 100
Where:
D. D is fluorescence at the emission wavelength 645 nm in the experimental cell sample, labeled with calcein AM and EthD-III.
E. E is fluorescence at the emission wavelength 645 nm in a sample where all the cells are dead, labeled with calcein AM only.

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F. F is fluorescence at the emission wavelength 645 nm in a sample where all the cells are dead, labeled with EthD-III only.
Optionally, to subtract background fluorescence, measure the fluorescence of the cellfree control at the emission wavelength 645 nm. Then, subtract the background fluorescence measured at the emission wavelength 645 nm from values D, E, and F.

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