ABSbio™ LIVE/DEAD Viability/Cytotoxicity Detection Kit

(Cat# K050-100; 100 assays; store kit at -20℃)

Introduction

Cell viability characteristics include cellular metabolic activity and cell membrane integrity. The measurement and monitoring of cell viability is an essential technique in any laboratory focused on cell-based research. The ABSbio™ LIVE/DEAD Viability/Cytotoxicity Detection Kit provides a two color fluorescence cell viability assay that is based on the simultaneous determination of live and dead cells with two probes that measure two recognized parameters of cell viability — intracellular esterase activity and plasma membrane integrity. The kit is suitable for use with fluorescence multi-well plate reader or fluorescence microscope and easily adaptable for use with flow cytometers and other fluorescence detection systems. The assay principles are general and applicable to most eukaryotic cell types, including adherent cells and certain tissues, but not to bacteria or yeast. This fluorescence based method of assessing cell viability can be used in place of 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.

Kit Components (100 tests)

Live cell Staining Dye:	**0.05 mL**	**Dead Cell Staining Dye:**	**0.05 mL**	**Saponin:**	**0.1 mL**

Storage and Handling: Store kit at -20℃. Shelf Life: 12 months after receipt. Warm up reagents to room temperature before use.

Protocol

1. Preparation of Reagents

Allow all reagents to warm to room temperature before use. Centrifuge all vials briefly prior to opening.

- Dye Solution: Prepare a 1X Dye solution by diluting the provided stocks 1:500 in medium. For example, to prepare a 5.0 mL solution, add 10 µL of **Live cell Staining Dye** and 10 µL of **Dead Cell Staining Dye** to 4.980 mL of medium. Vortex thoroughly. Note: Prepare only the amount necessary for the immediate application. Do not store diluted dye solutions.
- 1X Saponin Solution: Prepare a 1X Saponin solution by diluting the provided stock 1:100 in media. Vortex thoroughly. Store the diluted solution at 4℃.

2. Cell Culture and Treatment

- Add 10,000 to 50,000 cells per well to a black clear bottom 96-well cell culture plate. Culture the cells 12-24 hours at 37℃ and 5% CO_2. The time and culture conditions will depend on the cell line used and may need to be adjusted.
- Treat cells with compounds of interest, if desired. As a control, we recommend treating cells with vehicle alone. Add 1X Saponin solution to positive control wells to initiate cell death (100 µL/well in a 96- well plate). Incubate the plate for 10 minutes at room temperature. Carefully remove the media from the wells. Wash each well once with medium. Note: Saponin treated cells are very loosely attached to the plate. Gentle washing is essential to minimize cell loss.
- We recommend using suspension cells for flow cytometry application.

3. Cell Staining

For suspension cells, collect ~1 x 10^5 cells by centrifugation at 500 X g for 5 min. Resuspend in 0.1 ml Staining Solution. For adherent cells, remove the media carefully and add 0.1 ml Staining Solution to each well. Incubate for 15 to 60 minutes at 37℃, protected from light. *The appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.*

4. Detection or Measurement

Fluorescence reader: The live cell signal was measured at EX 485 nm/ EM 535 nm, the dead cell signal at EX 535 nm/ EM 635 nm. Either single measurements (one measurement point per well) or multiple reads per well (circle, 4x4; for geometrical view) were performed with the bottom reading mode of the instruments. The fluorescence in blank control wells with the growth medium is subtracted from the values for those wells with cells treated with the test compounds. The background fluorescence of the blank wells may vary depending on the sources of the microtiter plates or the growth media.

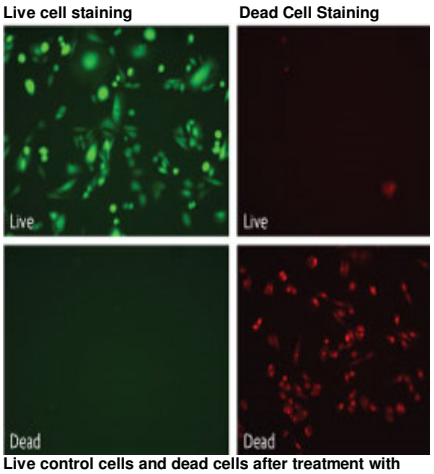
% Live Cell = $(RFU_{sample}-RFU_{blank})/(RFU_{control}-RFU_{blank})$ x 100%;

% Dead Cell = $(RFU_{sample}-RFU_{blank})/(RFU_{control}-RFU_{blank})$ x 100%

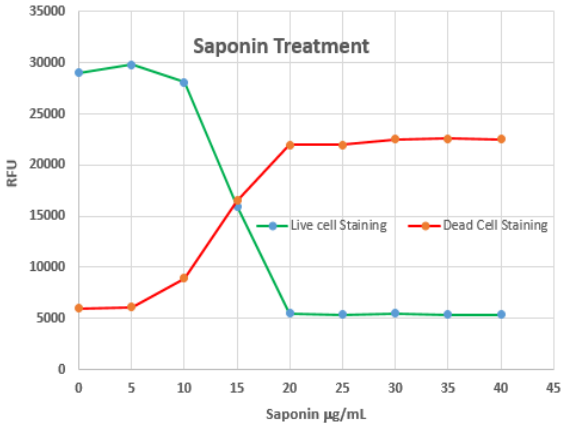
RFU_{sample}, RFU_{blank}, $RFU_{control}$ are related Fluorescent unit from treated sample, media only and untreated cell control.

Microscopy: For analyzing adherent cells, cell culture plates can be used directly. Place the cell suspension on a glass slide. Cover the cells with a glass coverslip. Observe cells immediately under a light and fluorescence microscope (detects green and red wavelength [Ex/Em = 485-495/530-635 nm]). Live Cell Staining Dye stains healthy cells green. Dead Cell Staining Dye stains dead cell red. Acquire several images per well for analysis.

Flow Cytometry: Resuspend cell pellet in HHBS or PBS (~10^5 cells/ml). Analyze immediately using flow cytometry. Live Cell Staining Dye is measured in the FL1 channel and Dead Cell Staining Dye is measured in the FL3 channel. To ensure that only proper target cells are gated, use a side scatter versus FL-1 plot.



Live control cells and dead cells after treatment with Saponin



Measurement of Cell staining after treated with Saponin

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References

Decherchi, P. et al. 1997, J Neuroscience Methods 71:205-213
Zhao, LQ et al. 2004, Brain Research. 1010:22-34

Related Products:

Cell Viability/Cytotoxicity Detection Kit (LDH based) (#K025-500)
Cell Viability/Cytotoxicity Detection Kit(WST-1 based) (#K010-500)