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Live/Dead Cell Viability Assay Kit (for Mammalian Cells-24 well)

rev 06/19

(Catalog # K502-100; 100 assays; Store at -20°C)

I. Introduction:

Quantification of number of live and dead cells is an indispensable tool in cell biology research. BioVision's Live/Dead Cell Viability Assay Kit, provides a two-color fluorescence method that is based on the simultaneous determination of live and dead cells using two different dyes. Live cell dye easily penetrates intact, live cells and intracellular esterase hydrolyzes the dye to produce a hydrophilic, strongly fluorescent compound that is retained in the cell cytoplasm which can be measured at Ex/Em = 485/530 nm. Dead cell dye enters damaged cell membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acid, thereby producing a bright red fluorescence (Ex/Em = 495/635 nm) in dead cells. This assay kit provides an easy-to-use, non-radioactive, histological and FACS-based method for measuring cell proliferation, cell viability, chemotaxis, cytotoxicity and apoptosis.

II. Application:

- Screening/studying/characterization of stimulators/inhibitors that affect cell viability.

III. Sample Type:

- Adherent cells or suspension cells.

IV. Kit Contents:

Components	K502-100	Cap Code	Part Number
Assay Buffer	100 ml	NM	K502-100-1
Live Cell Staining Dye	1 vial	Green	K502-100-2
Dead Cell Staining Dye	50 µl	Red	K502-100-3

V. User Supplied Reagents & Equipment:

- 6-well, 12-well, 24-well
- 37°C Incubator with 5% CO_2
- Light and fluorescence microscope with Ex/Em = 485-495/530-635 nm.
- FACS with Red and Green Channel detector.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Briefly spin the small vials prior to use. Read entire protocol before performing the assay. Open all reagents under sterile conditions (e.g. cell culture hood).

- **Assay Buffer:** Store at 4°C or -20°C. Warm to 37°C before use.
- **Live Cell Staining Dye:** Reconstitute in 100 µl DMSO. Light sensitive, do not expose to intense light. Store at -20°C.
- **Dead Cell Staining Dye:** Light sensitive, do not expose to intense light. Store at -20°C.

VII. Live/Dead Cell Viability Assay Protocol:

This protocol is for a 24-well plate. Adjust the volume according to the plate size.

1. Cell Culture and Staining:

a. Grow cells in 37°C incubator containing 5% CO_2 in desired media. Treat cells with compounds of interest, if desired. As a control, we recommend treating cells with vehicle alone.

Notes:

- Adherent cells can be grown on cover slip for microscopy application to obtain better image resolution.
- We recommend using suspension cells for flow cytometry application.

b. Mix 2 µl of Live Cell Staining Dye and 1 µl of Dead Cell Staining Dye in 1 ml of Assay Buffer. Prepare enough Staining Solution for your assay (0.5 ml per well in 24 well dish). Scale up accordingly for larger numbers of assays.

c. For suspension cells, collect ~1 x 10^6 cells by centrifugation at 500 X g for 5 min. Resuspend in 0.5 ml Staining Solution. For adherent cells, remove the media carefully and add 0.5 ml Staining Solution to each well. Incubate for 15 min. at 37°C.

2. Detection:

a. Microscopy: Place the cell suspension on a glass slide. Cover the cells with a glass coverslip. For analyzing adherent cells, cell culture plates can be used directly. If cells are grown on a coverslip, invert coverslip on a glass slide and visualize cells. 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.

b. Flow Cytometry: Wash cells once with PBS. Resuspend cell pellet in Assay Buffer (~10^6 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.

Notes:

- We recommend staining cells with Live Cell Staining Dye alone and Dead Cell Staining Dye alone to choose the proper instrument gating set up.
- We recommend keeping unstained control cells (i.e. without any Dye staining) suspended in Assay Buffer for both treated and untreated samples to set up the flow cytometer instrument.

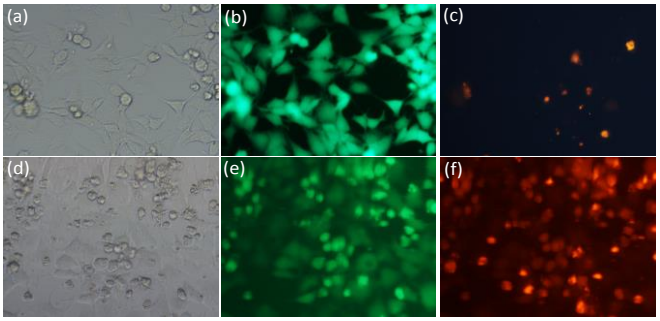

Figure 1: Analysis of Live/Dead HeLa Cells by Microscopy: HeLa cells were cultured overnight with (d-f) or without (a-c) comptothecin (5 µM), which induces apoptosis. Next day, cells were treated with Staining Solution, as described in the protocol. Light and fluorescence images of cells were taken using Nikon TiE microscope. Treatment with comptothecin caused increased apoptosis in cells, which is demonstrated by increased number of dead cells, as shown in f.

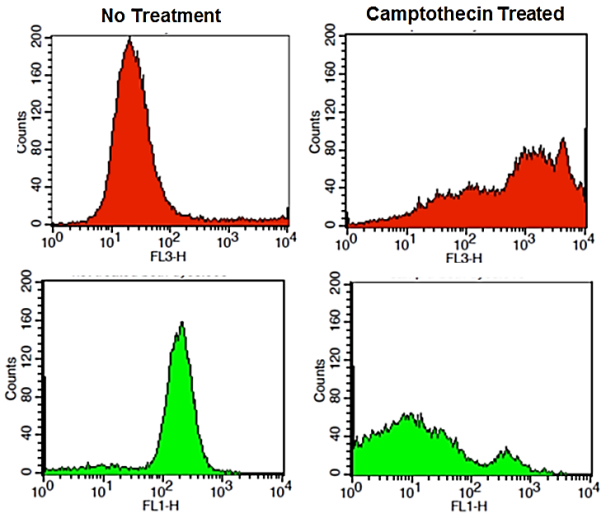

Figure 2: Analysis of Live/Dead Jurkat Cells by Flow Cytometry: Jurkat cells (10^6 cells/ml) were grown in RPMI media supplemented with 10% FBS. Cells were treated with or without camptothecin (5 µM) overnight. Next day, cells were stained with Staining Solution, as described in the protocol. The graph (right side) displays the cytotoxic effect of the compound, illustrating apoptosis using Dead (Red) and Live (Green) Cell Staining Dye. In control cells (No Treatment), majority of the cells were sorted in FL-1 Channel (Green) and few were in FL-3 (Red) channel. In treated cells, majority of the cells were sorted in FL-3 Channel (Red) and some cells were in FL-1 (Green) channel.

VIII. RELATED PRODUCTS:

BrdU Cell Proliferation Assay Kit (K306)
Quick Cell Proliferation Colorimetric Assay Kit (K301)
Ready-to-use Cell Proliferation Reagent, WST-1 (K304)
MTS Cell Proliferation Colorimetric Assay Kit (K300)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ATP Colorimetric Assay Kit II (K354)
WST-1 (2198)
Live-Dead Cell Staining Kit (K501-100)
Quick Cell Proliferation Colorimetric Assay Kit plus (K302)
VisionBlue™ Quick Cell Viability Fluorometric Assay Kit (K303)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ADP Colorimetric Assay Kit II (K356)
StayBrite™ Highly Stable ATP Bioluminescence Assay kit (K791)
MTT Cell Proliferation Assay Kit (Colorimetric) (K299)

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