ELISA PRODUCT INFORMATION & MANUAL

Cell Viability Assay Kit (Fluorometric)

NBP2-54867

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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NBP2-54867 – Cell Viability Assay Kit (Fluorometric)

For screening/studying/characterization of stimulators/inhibitors that affect cell viability.
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer	100 mL	-20°C
Live Cell Staining Dye	1 vial	-20°C
Dead Cell Staining Dye	50 µL	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 6-well, 12-well, or 24-well plate
- 37°C Incubator with 5% CO2
- Light and fluorescence microscope with Ex/Em = 485-495/530-635 nm
- FACS with Red and Green Channel detector

Reagent Preparation

- Read the entire protocol before performing the assay.
- Briefly spin the small vials prior to use.
- 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.

Assay Protocol

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

Cell Culture and Staining:

1. Grow cells in 37°C incubator containing 5% CO2 in desired media. Treat cells with compounds of interest, if desired. As a control, we recommend treating cells with vehicle alone.
   Δ Notes:
   a) Adherent cells can be grown on cover slip for microscopy application to obtain better image resolution.
   b) We recommend using suspension cells for flow cytometry application.
2. 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.
3. 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.

Detection:

Microscopy:

1. Place the cell suspension on a glass slide. Cover the cells with a glass coverslip.
2. 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.
3. 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.
4. Acquire several images per well for analysis.

Flow Cytometry:

1. Wash cells once with PBS.
2. Resuspend cell pellet in Assay Buffer (~10^6 cells/mL).
3. 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.
4. To ensure that only proper target cells are gated, use a side scatter versus FL-1 plot.

Δ Notes:

a) *We recommend staining cells with Live Cell Staining Dye alone and Dead Cell Staining Dye alone to choose the proper instrument gating set up.*

b) *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.*