ION Vital - Viability

Kit Contents (Cat # 5000)

Label	Name	Size	Quantity	Storage
Reagent A	Ethidium homodimer I (EthD-I), 2 mM in DMSO/H_2O[1]	300 µL Vial	1	-20° C
Reagent B	Calcein AM, 4 mM in DMSO[2]	100 µL Vial	1	-20° C

Description

ION Vital - Viability assay kit is a simple, rapid, and versatile solution for detecting live (green) and dead (red) cells within a population. ION Vital - Viability is compatible with fluorescence microscopy, flow cytometry, and plate reader applications.

Calcein AM is a membrane-permeant, non-fluorescent form of calcein that enters cells passively. Once inside the cytosol of cells, Calcein AM is converted to green-fluorescent Calcein by ubiquitous esterases in viable cells, resulting in uniform cytosolic fluorescence (Ex/Em 495 nm/515 nm). Calcein, a polyanionic dye, is membrane-impermeant and is well retained within the cytosol of healthy cells with intact cell membranes.

Ethidium homodimer I (EthD-I) is a membrane-impermeable, high-affinity, nucleic acid stain that is excluded by viable cells with intact cell membranes. When membrane integrity is compromised, a hallmark of dead or dying cells, EthD-I enters the cell and binds DNA, which results in a >30-fold enhancement in bright red, nuclear fluorescence (Ex/Em 528 nm/617 nm).

ION Vital—Viability can be used to quantify live and dead cells within eukaryotic cell suspensions or adherent cultures, 3D cultures, organoids, and some non-fixed tissues, but cannot be used for yeast or bacteria.

When following our protocol, ION Vital—Viability kit provides enough reagents to make 150 mL of working solution, enough for fifteen 96-well plates or 1,500 flow cytometry samples. The actual number of assays will vary according to optimal dye concentrations for your application.

Laboratory Procedures

Getting Started

Before you begin, make sure that you have all the additional reagents and materials you will need for the successful completion of your experiment. Although the ION Vital - Viability kit contains the key reagents you will need to prepare your cells for analysis, your experiments will likely require other reagents which are not included in your ION Vital - Viability package. Notably, compounds to be tested, buffers and solvents for the dissolution of these compounds, or reagents necessary for cell culture are not included.

In addition to reagents, a microscope, fluorescence plate reader, or flow cytometer that is capable of providing an excitation source at ~ 490 nm and ~520 nm and measuring emission at ~530 nm and ~620 nm is required.

General Considerations

1. Optimal dye concentrations will vary depending on cell type and application. Recommended dye concentrations range between 0.1 µM and 10 µM.
2. Aqueous solutions of Calcein AM are susceptible to hydrolysis; therefore, all working solutions should be used as quickly as possible and no later than 24 hours after preparation.
3. Serum-containing preparations will increase extracellular Calcein fluorescence. When possible, A wash step can remove extracellular fluorescence.
4. Calcein cannot withstand fixation after staining.
5. Dead cell controls can be prepared by applying 0.1% saponin or 0.1 - 0.5% digitonin to live cells for 10 min.
6. Cytotoxic events that do not affect esterase activity or membrane permeability are not going to be accurately measured using this kit.

Plate Reader Assay

1. Seed cells in a 96-well (or 384-well) plate and treat with test compounds of your choosing prior to staining.
2. Remove stock solutions of EthD-I and Calcein AM from freezer and allow to warm to room temperature.
3. Prepare dead cells by treating with 0.1% saponin or digitonin solutions for 10 min.
4. Prepare a working solution that contains 4 µM EthD-I (Reagent A, 1:500 dilution) and 2 µM Calcein AM (Reagent B, 1:2000 dilution) in phosphate buffered saline (PBS) or other serum free medium or buffer. For example, add 20 µL of EthD-I (Reagent A) and 5 µL of Calcein AM (Reagent B) to 10 mL of PBS. Vortex briefly to mix.
5. Optional: Wash the cells with serum-free buffer or medium to remove serum. For suspension cells, use a centrifuge to pellet cells, then resuspend in 100 µL of serum free medium or buffer. The wash solution can be aspirated from wells prior to the addition of working solution if desired.
6. Add working solution prepared in step 4 directly to cells. We recommend 100 µL/well for a 96-well plate.
7. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
8. Measure fluorescence using a microplate reader. For Calcein, use Ex/Em ~485 nm/520 nm or FITC settings. For EthD-I, use Ex/Em ~530 nm/620 nm or Texas Red® settings.

Note: Absolute numbers of viable and dead cells can be estimated by generating standard curves. Standard curves are prepared by seeding known densities of healthy and dead cells in wells prior to staining. Standard curves should be created for both viable and dead cells. Dye fluorescence intensity is linearly related to the number of live or dead cells as demonstrated in Example Results.

Microscopy Assay

1. Remove stock solutions of EthD-I and Calcein AM from freezer and allow to warm to room temperature.
2. Prepare a working solution that contains 4 µM EthD-I (Reagent A, 1:500 dilution) and 2 µM Calcein AM (Reagent B, 1:2000 dilution) in phosphate buffered saline (PBS) or other serum free medium or buffer. For example, add 20 µL of EthD-I (Reagent A) and 5 µL of Calcein AM (Reagent B) to 10 mL of PBS. Vortex briefly to mix.
3. Optional: Wash the cells with serum-free buffer or medium to remove serum.

4. Add sufficient volume of the working solution prepared in step 2 to completely cover cells.
5. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
6. Optional: Replace working solution with fresh buffer or medium prior to imaging.
7. Image stained cells using fluorescence microscopy. For Calcein, use a GFP or FITC filters. For EthD-I, use a Texas Red®, propidium iodide (PI), or rhodamine filters.

Flow Cytometry Assay

1. Remove stock solutions of EthD-I and Calcein AM from freezer and allow to warm to room temperature.
2. Prepare a working solution that contains 4 µM EthD-I (Reagent A, 1:500 dilution) and 2 µM Calcein AM (Reagent B, 1:2000 dilution) in phosphate buffered saline (PBS) or other serum free medium or buffer. For example, add 20 µL of EthD-I (Reagent A) and 5 µL of Calcein AM (Reagent B) to 10 mL of PBS. Vortex briefly to mix.
3. Pellet cells via centrifugation, remove supernatant, then resuspend in 100 µL of serum free medium or buffer.
4. Add 100 µL of working solution prepared in step 2 directly to cells.
5. Incubate cells for 30 - 45 min at room temperature or 37°C. Protect from light.
6. Pellet cells again via centrifugation and resuspend in preferred flow cytometry buffer.
7. Analyze cells using a flow cytometer. To detect calcein (+) cells, use FITC settings. To detect EthD-I (+) cells, use phycoerythrin (PE) or PE-Texas Red® settings. Use single color stained cells to perform standard compensation.

Example Results

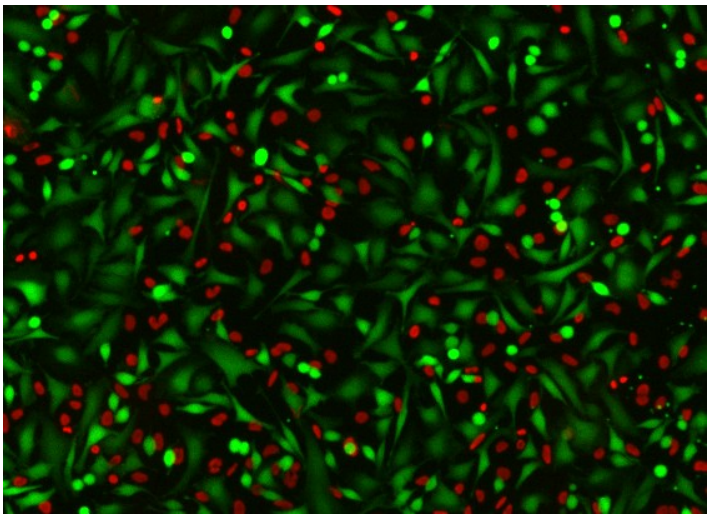

Figure 1. Fluorescence image of live (green) and dead (red) cells stained using ION Vital - Viability kit. Image acquired using a 4X objective with GFP (calcein) and propidium iodide (EthD-I) filter cubes.

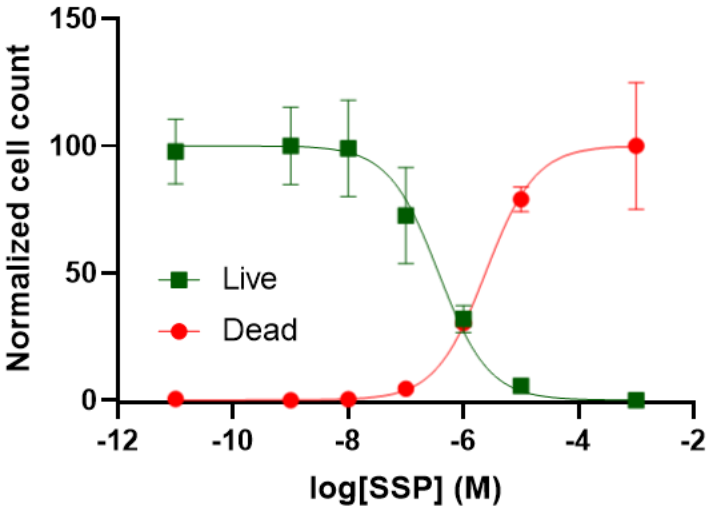

Figure 2. Cytotoxicity screen using ION Vital Viability kit of HeLa cells treated with various concentrations of staurosporine (SSP), a known cytotoxic agent, for 24 hrs. N = 3 wells for all data points.

Laboratory Procedures - Footnotes

[1] Calcein AM, 4 mM in DMSO is not a regular catalog item available for purchase, however, ION Biosciences does stock a 2 mM in DMSO solution for sale (see Table 1 below), which can be used in place of Reagent B by simply doubling the volume used in any of the procedures described in this protocol.

[2] Caution is advised when using TRS as it may have undesirable effects on assay performance for the target of interest. TRS contains a membrane-impermeant dye useful for masking extracellular fluorescence.

Additional Information

Additional dye indicator and buffer reagents can be purchased either directly from our website or by contacting our Sales Department.

Table 1	Additional Reagents	Available Sizes	
Kit Label	Name	Size	Catalog #
Reagent A	Ethidium Homodimer I, 2 mM in DMSO/H_2O	0.5 mL x 1 Vial	5020
Alternative to Reagent B	Calcein AM, 2 mM in DMSO	0.5 mL x 1 Vial	5030