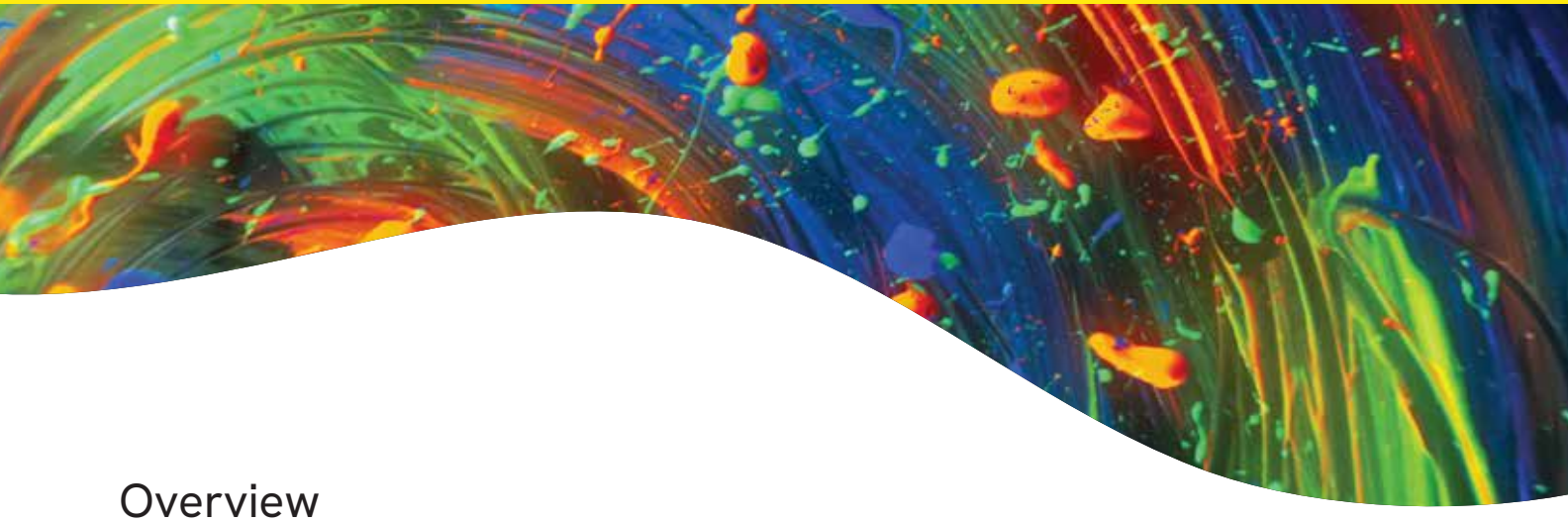
PhenoVue Live/Dead Cell Viability Assay Kit



Overview

PhenoVue™ live/dead cell viability assay kit provides a two-color fluorescence method which enables the simultaneous determination of live and dead cells using two different dyes. Calcein AM dye penetrates live cells where intracellular esterases hydrolyze the dye to generate a bright green, fluorescent signal. Propidium Iodide dye enters damaged cell membranes of dead cells and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acid, thereby producing a bright red fluorescence. This assay kit provides an easy-to-use method for measuring cell proliferation, cell viability, cytotoxicity and apoptosis.

Product information

Product name	Part no.	Number of vials/bottles per kit	Shipping conditions
PhenoVue live/dead cell viability kit	PCVA11	5	Dry ice

Kit contents	Format	Quantity	Storage
Calcein-AM	Lyophilized	2 vials	≤ -16 °C or below.
Propidium iodide	Liquid	1 vial (40 µL)	≤ -16 °C or below.
DMSO	Liquid	1 vial (100 µL)	≤ -16 °C or below.
Assay buffer	Liquid	1 bottle (20 mL)	≤ -16 °C or below.

Storage and stability

- Store the kit at ≤ -16 °C or below, protect from light exposure.
- The stability of the kit is guaranteed until the expiration date provided in the Certificate of Analysis, when stored as recommended and protected from light.

Equivalent number of microplates

Product name	96-well microplate (100 µL per well)	384-well microplate (25 µL per well)	1536-well microplate (4 µL per well)
PhenoVue live/dead cell viability kit	2	2	3

View our full range of high-quality imaging microplates at Revvity.com

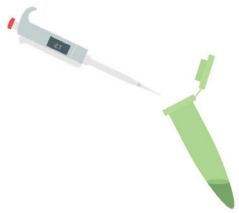
Preparation of stock and working solutions

Calcein-AM stock solution	Calcein-AM/propidium iodide working solution
To the vial of Calcein-AM, add 20 µL DMSO and homogenize. Protect from light.	To 10 mL of assay buffer, add: - 20 µL of Calcein-AM stock solution previously prepared - 20 µL of propidium iodide Mix the working solution. This solution is stable for at least 2 hours at room temperature.

Example preparation of solutions

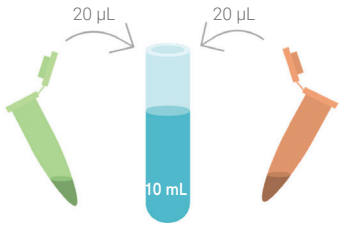
The following example describes the preparation of 10 mL working solution, sufficient for 1 x 96-well plate (100 µL/well) or 1 x 384-well plate (25 µL/well).

① Calcein-AM stock solution



Add 20 µL DMSO into Calcein-AM lyophilized vial

② Calcein-AM/propidium iodide working solution



In 10 mL assay buffer, add
- 20 µL of Calcein-AM stock solution
- 20 µL of propidium iodide

Spectral and photophysical properties

Product name	Maximum excitation wavelength (nm)	Maximum emission wavelength (nm)	Filter set
Calcein-AM	501	521	FITC
Propidium iodide	537	618	TRITC

Live- and fixed-cell compatibility

Product name	Live-cell staining	Fixation/permeabilization steps post live-cell staining	Fixed-cell staining
PhenoVue live/dead cell viability kit	Yes	No	No

Protocol

1. Treat cells with desired compounds under 100 µL final volume per well of 96-well plate or 25 µL final volume per well of 384-well plate.
2. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of Calcein-AM/propidium iodide working solution.
3. Incubate the plate at room temperature or 37 °C for 30 minutes to 1 hour, protected from light.
   - **Note** that the optimal incubation time depends on the cell type and cell concentration used. This should be determined accordingly.
   - **DO NOT** wash the cells after loading the working solution. For non-adherent cells, it is recommended to centrifuge cell plates at 800 rpm for 2 minutes with brake off after incubation.
4. Acquire dual fluorescent images on a Revvity high-content screening system or other automated imaging microscopy system using FITC filter set for live cells and Cy3/TRITC filter for dead cells.

Safety information

Chemical reagents are potentially harmful, please refer to the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Validation data

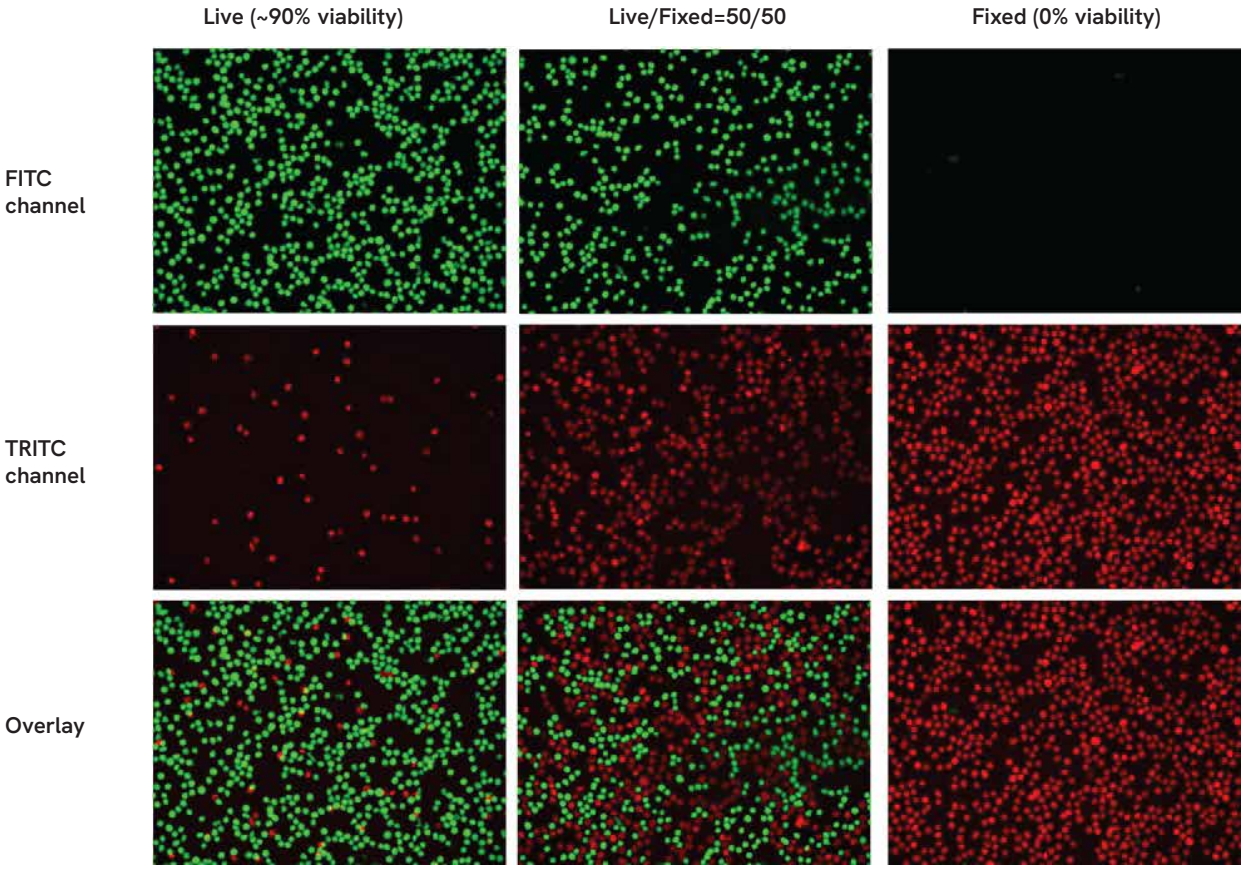


Figure 1: 90% viability cells (live cells), 0% viability cells (fixed cells) and the mixture of two cells (live/fixed=50/50) were analyzed with PhenoVue live/dead cell viability kit and imaged in FITC and TRITC channels with fluorescence microscope.

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