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Directions for Use
Cytus™ Green / Red Cell Viability Kit

Cat # 5471-1KIT

Product Overview

The Cytus™ Green / Red Cell Viability Kit is a dual-color fluorescence staining solution designed for rapid and reliable assessment of live and dead cells, analogous to standard Live/Dead viability assays. The kit combines Cytus™ Green , a calcein AM–based stain for viable cells, with Cytus™ Red, a propidium iodide (PI)–based stain for non-viable cells, enabling clear and simultaneous discrimination within mixed cell populations.

Cytus™ Green (calcein AM) is a cell-permeable, non-fluorescent compound that is enzymatically converted into intensely green fluorescent calcein by intracellular esterases in metabolically active cells (Ex/Em = 501/521 nm). Because this conversion requires intact cell membranes and active esterases, fluorescence is specific to live cells, with signal intensity correlating linearly to viable cell number. The resulting hydrophilic calcein is retained within the cytoplasm, providing strong, stable fluorescence suitable for quantitative viability and cytotoxicity measurements.

Cytus™ Red (propidium iodide) selectively labels dead or membrane-compromised cells by intercalating into nucleic acids, producing a strong red fluorescent signal upon binding (Ex/Em = 537/617 nm). PI fluorescence increases dramatically when bound to DNA or RNA, making it a sensitive marker for cell death and a widely accepted counterstain in multicolor assays. Together, Cytus™ Green and Cytus™ Red enable robust viability imaging and are compatible with fluorescence microscopy, flow cytometry, microplate readers, and fluorometric analyses.

Kit components

Components	Specification: 500 tests	Storage
Cytus™ Green Cat No. 5468-100UL	1 mM in DMSO	-20 °C
Cytus™ Red Cat No. 5467-100UL	1 mM in DMSO	-20 °C

Materials Needed

- Cytus™ Green/ Cytus™ Red Cell Viability Kit (Cat #5471-1KIT)
- Cells/cell lines of interest
- Cell culture media
- Sterile 1X PBS
- Cell culture plates
- Centrifuge tubes
- Serological pipettes
- Micropipette and tips
- Orbital shaker
- Fluorescence microscope

Sample Procedure for Staining Cells

Note: The optimal concentrations of Cytus™ Green and Cytus™ Red vary depending on the cell type used. Dye concentrations should be adjusted for specific cells to achieve the optimal staining performance. In general, the lowest possible dye concentration should be selected when sufficient signal intensity is obtained without over-saturation. The recommended concentration range for Cytus™ Green is 2–10 µM and Cytus™ Red is 2–5 µM.

*Ensure use of the appropriate PPE when performing the following steps:

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1. Seed and culture cells according to manufacturer's protocol for desired time.

2. Remove cell culture medium and wash/rinse the cell monolayer with sterile 1 × PBS twice.

3. Thaw Cytus™ Green and Cytus™ Red at room temperature until the stock solution completely turns into liquid.

4. If necessary, centrifuge the cryovials to collect the dyes at the bottom of their respective containers.

5. **Prepare Staining Working Solution (2 µM Cytus™ Green, 2 µM Cytus™ Red):**

   (1) Add 20 µL **Cytus™ Green** stock in 10 mL 1X PBS to obtain a 2 µM Cytus™ Green working solution.

   (2) Add 20 µL **Cytus™ Red** stock in 10 mL 1X PBS to obtain a 2 µM Cytus™ Red working solution.

6. **Staining**: aspirate the PBS in cell culture wells, add sufficient **Cytus™ Red working solution** to cover the wells and incubate the plates protected from direct light at room temperature for 10 minutes.

Table 1. Recommended volume of working solution for each well plate type.

Well Plate Type	Volume of Working Solution per Well
6-Well	1 to 3 mL
12-Well	1 to 2 mL
24-Well	0.5 to 1 mL
48-Well	200 to 400 µL
96-Well	100 to 200 µL

7. Remove the **Cytus™ Red working solution** and rinse the stained wells thoroughly with 1X PBS twice.

8. Add sufficient **Cytus™ Green working solution** to cover the wells and incubate. Reference Table 1 for volume estimates. Keep the plates protected from direct light and incubate at room temperature for 20 minutes.

9. Remove the **Cytus™ Green working solution** and rinse the stained wells thoroughly with 1 × PBS twice.

10. Cover stained cells with 1 × PBS and load the plate for imaging.

11. **Imaging:** Image the stained plates using the GFP and TexasRed (or mCherry) channels.

Note: Cytus™ Green is prone to hydrolysis and its working solution should be used on the same day they are prepared. We recommend completing data acquisition and analysis within 6 hours after staining.

Sample images

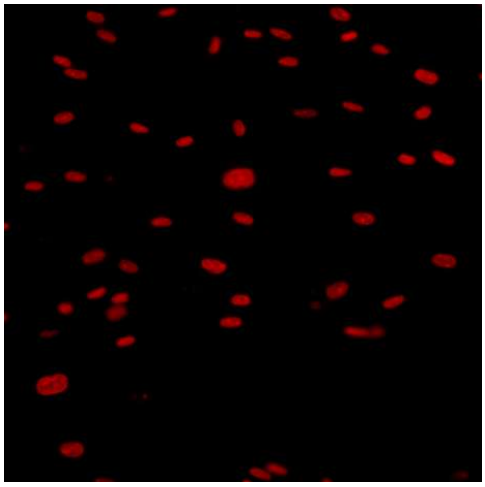

Fig.1. Nonviable human fibroblasts stained with Cytus™ Red.

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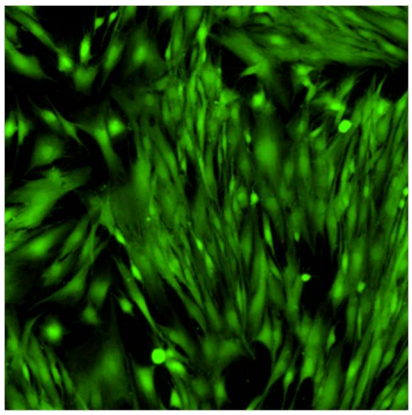

Fig.2. Viable human fibroblasts stained with Cytus™ Green.

