TransDetect® Cell LIVE/DEAD Viability/Cytotoxicity Detection Kit

Cat. No. FC301

Storage: at -20°C in the dark for up to one year.

Description

The *TransDetect*® Cell LIVE/DEAD Viability/Cytotoxicity Detection Kit provides a simple, high-specificity and low-toxicity way to distinguish between live and dead cells by combining Calcein-AM and Propidium iodide (PI). Quantification or sorting can be performed by flow cytometry.

Calcein-AM is a low-cytotoxicity non-fluorescent dye, which easily permeates intact live cells. In live cells, the Calcein-AM is converted to green fluorescent calcein with excitation and emission wavelengths of 490/515 nm by intracellular esterase digestion.

PI is excluded by intact plasma membrane of live cells. It is a DNA-binding fluorescent dye with excitation and emission wavelengths of 535/617 nm， producing a bright red fluorescence in dead cells with broken membrane.

- Higher specificity.
- More simplicity.
- Lower cytotoxicity.

Kit Contents

Component	100 rxns
Calcein-AM Solution (1000×)	100 µl
PI Solution (1000×)	100 µl

Procedures

Materials required but not included:

Product Name	Catalog
PBS without calcium or magnesium	TransGen, Cat. FG701-01

Preparation of staining working solutions

1. Remove Calcein-AM Solution (1000×) and PI Solution (1000×) from the refrigerator and allow them to warm to room temperature before use. It is recommended to aliquot the solution into small volumes to avoid repeated freeze-thaw cycles before the first use.
2. Preparation of double staining working solutions: add 1 µl of Calcein-AM Solution (1000×) and 1 µl of PI Solution (1000×) to 1 ml of 1×PBS. Vortex thoroughly.
3. Preparation of single staining working solution: add 1 µl of Calcein-AM Solution (1000×) or 1 µl of PI Solution (1000×) to 1 ml of 1×PBS. Vortex thoroughly.

Note: Optimal results are likely to vary depending on cell types. It is recommended to adjust dilution ratios (200×-5000×) of Calcein-AM and PI in a single staining working solution for first-time use.

Dead cells can be prepared according to the following methods: cells are fixed with 4% paraformaldehyde for 10 minutes, and then permeabilized with 0.3% Triton-X-100 for 5 minutes; or cells are treated with 0.1% saponin or 0.1-0.5% digoxin for 10 minutes.

Staining

1. Suspension staining method
   (1) Prepare 5×10^5~1×10^6 adherent cells. Trypsinize the cells and centrifuge at 200×g for 5 minutes. Collect the cells by discarding the supernatant. For suspended cells, just centrifuge.
   (2) Add 1 ml of 1×PBS to resuspend the cells and centrifuge at 200×g for 5 minutes. Discard the supernatant. Repeat once.
   (3) Add 500µl-1ml of double staining working solutions to resuspend the cells. Incubate for 15–20 minutes at 37°C in the dark.
   (4) Centrifuge the cells at 200×g for 5 minutes. Discard the supernatant.
   (5) Add 1 ml of 1×PBS to resuspend the cells and centrifuge at 200×g for 5 minutes. Discard the supernatant. Repeat 1-2 times.

(6) Add 500 µl of 1×PBS to resuspend the cells and keep in the dark. It is recommended to detect within 1 hour.

This method is suitable for flow cytometry or fluorescence microscopy.

2. Adherent cells staining method

(1) Seed cells in 24-well plates at a density of 2×10^5 cells/ml, and incubate overnight.

(2) Remove growth medium and wash the cells with 1×PBS for 1-2 times. Discard the PBS.

(3) Add 500µl of double staining working solutions. Incubate for 15–20 minutes at 37°C in the dark.

(4) Wash the cells with 1×PBS for 1-2 times and keep in the dark. It is recommended to detect with in 1 hour.

This method is suitable for fluorescence microscopy.

Sample analysis

A. Analysis by Flow Cytometry

Choose appropriate voltage and adjust fluorescence compensation for flow cytometry analysis. It is recommended to set the following control cells in addition to experimental cells.

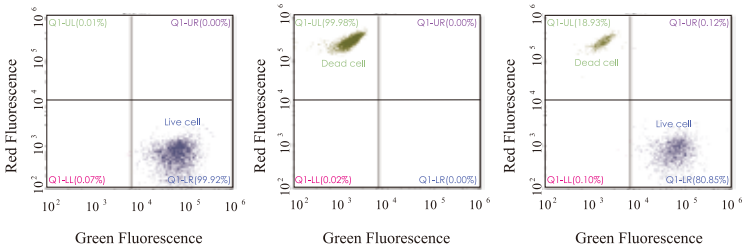
(1) Unstained negative control cells

(2) Calcein-AM single color stained positive cells.

(3) PI single color stained positive cells.

Example

A 4:1 mixture of live and fixed-permeabilized HeLa cells was double stained according to the protocol provided. As is shown in the following figure, cell population was separated into two groups: live cells with green fluorescence and dead cells with red fluorescence.



B. Analysis by Fluorescence Microscopy

(1) Suspension cells

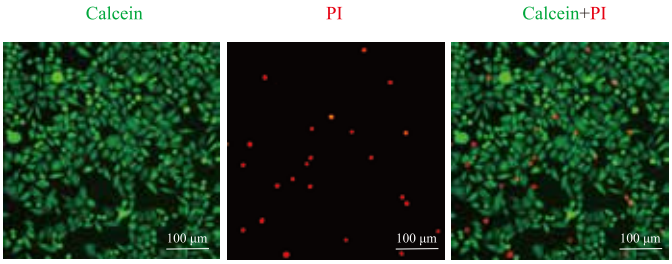
Transfer an aliquot of the stained cell suspension onto a glass slide, cover the coverslip and observe samples by fluorescence microscopy. Live cells can be labeled with Calcein-AM (Ex=490 nm, Em=515 nm) showing green fluorescence, and dead cells can be labeled with PI (Ex=535 nm, Em =617nm) showing red fluorescence.

(2) Adherent cells

Adherent cells can be cultured on coverslips and directly stained for fluorescence microscopy analysis. The cells can also be trypsinized, stained, and then partially transferred onto a coverslip for fluorescence microscopy analysis. Live cells can be labeled with Calcein-AM (Ex=490 nm, Em=515 nm) showing green fluorescence, and dead cells can be labeled with PI showing red fluorescence (Ex=535 nm, Em =617nm).

Example

Hep G2 cells were stained according to the provided protocol. As is shown in the following figure, live cells showing green fluorescence and dead cells showing red fluorescence can be distinguished through fluorescence microscopy analysis.



Notes

a. The staining working solutions should be freshly prepared. It is suggested to finish the detection within 1 hour after staining.

b. For your health, please wear gloves and standardize experimental operation.

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