Cat. No. 295

V.2023.10.13

Dr. View

Live/Dead dual-staining kit for mammalian cells

Product Information

Quick Facts

1. **Storage conditions before opening:<-20 ℃/ Protect from light.**
2. **Ex/Em (nm)：live cell (485/530)、Dead cell (530/645).**
3. **Recommended dye concentration for staining working solution：**
   2 μg/mL GL & 10 μg/mL RD (Dilution ratio：2000X)。
4. **The mixing ratio of cell sample and working solutions：1 / 1.**

Introduction

This live/dead dual-staining assay kit provides a two color fluorescence cell viability assay that is based on the simultaneous determination of live and dead mammalian cells with two probes that measure parameters of cell viability- intracellular esterase activity and plasma membrane integrity. The kit is suitable for use with fluorescence microscopes , fluorescence microplate reader, flow cytometers and other fluorescence devices. This product can be used in most eukaryotic cells including adherent cells and certain tissues, but not to bacteria or yeast.

Shipping and Storage Information

Product Name	shipping	Storage
Dr. View (Cat. No.：295)	under-20 °C with protection from light.	12 months under-20 °C with protection from light.

Handling of Reagents

1.Allow the reagents to warm to room temperature (about 30 mins) and centrifuge briefly before opening. Before refreezing, seal all stock solutions tightly.

2.GL fluorescent dye is sensitive to moisture and light. Prepare aqueous working solution immediately prior to use, and use it right away. RD is stable. The working solution of RD can stored at -20 °C about one year.

Kit Contents (1000 tests)

1. Dr. View GL stock solution for live cell (Green label, brown vials)：
   Two vials, 4 mg/mL, 50 μL each.
2. Dr. View RD stock solution for dead cell (Red label, brown vials)：
   Two vials, 20 mg/mL, 50 μL each.

Protocol

1. Fluorescence microscopes：

1.1 Select the Optical Filters：

The stained live and dead cells can be viewed simultaneously or separately. Typical characteristics of some appropriate filters are summarized in the following table：

	Omega Filters	Chroma Filters
Simultaneous viewing of live and dead cells	XF25, XF26, XF115	11001, 41012, 71010
Viewing live cell only	XF22, XF23	31001, 41001

Viewing dead cell only	XF32, XF43, XF102, XF108	31002, 31004, 41002, 41004

1.2 Prepare the Cells：

a. Adherent cells may be cultured on sterile glass coverslips as either confluent or subconfluent monolayers (e.g., fibroblasts are typically grown on the coverslip for 2–3 days until acceptable cell densities are obtained). The cells may be cultured inside 35 mm disposable petri dishes or other suitable containers; non-adherent cells may also be used.
b. Wash the cells prior to the assay to remove or dilute serum esterase activity generally present in serum-supplemented growth media (serum esterases could cause some increase in extracellular fluorescence by hydrolyzing calcein AM). Wash adherent cells gently with 500–1,000 volumes of Dulbecco' s phosphate-buffered saline (D-PBS)。
c. Wash non-adherent cells in a test tube with 500–1,000 volumes D-PBS and sediment by centrifugation. Transfer an aliquot of the cell suspension to a coverslip. Allow cells to settle to the surface of the glass coverslip at 37°C in a covered 35 mm petri dish.
d. Treat the cells with cytotoxic agents as required at any time prior to Live/Dead dual-staining.

1.3 Dilution Protocol：10 mL of working solution (This is an example; the optimal dye concentrations are likely to vary depending on the cell type.)

a. Allow the reagents to warm to room temperature (about 30 mins) and centrifuge briefly before opening.
b. Add 5 µL of the supplied **RD** stock solution to 10 mL of sterilized D-PBS, vortexing to ensure mixing thoroughly.
c. Combine the reagents by transferring 5 µL of the supplied **GL** stock solution to the above 10 mL **RD** working solution. Vortex the resulting solution to ensure mixing thoroughly.
d. The above working solution results approximately 2 µg/mL **GL** and 10 µg/mL **RD**, then, added directly to cells. This working solutions should therefore be used within 2 hours.

1.4 Perform the Viability Assay：

a. Add 100–150 µL of the combined assay working solution (procedure **1.3**) to the surface of a 22 mm square coverslip (procedure **1.2**), so that all cells are covered with solution. Incubations should be performed in a covered dish (e.g., 35 mm disposable petri dish) to prevent contamination or drying of the samples. Incubate the cells for 30–45 minutes at room temperature.
b. Following incubation, add about 10 µL of the fresh working solution or D-PBS to a clean microscope slide. Using fine-tipped forceps, carefully and quickly invert and mount the wet coverslip (procedure **1.4a**) on the microscope slide. To prevent evaporation, seal the coverslip to the glass slide e.g., with clear fingernail polish. Avoid damaging or shearing cells in the preparation of the slides.
c. View the labeled cells under the fluorescence microscope.

1.5 Problems and solutions：

a. **Prepare dead cells**：
Before staining the cells, if necessary, you can divide the cells into several aliquots, and

killed one of the aliquots cell with one of the following methods：0.1% saponin for 10 minutes, 0.1–0.5% digitonin for 10 minutes, 70% methanol for 30 minutes.

b. Determine the Optimal Dye Concentrations：
The optimal dye concentrations are likely to vary depending on the cell type. If the above method (procedure **1.3**) is not suitable for your cells, please try the following methods to determine the best operating dye concentration (you can carry out either at room temperature or at a constant temperature, such as 37 °C):
(1) Stain dead cells：Using dead cells samples, select an **RD** concentration that stains the dead cell nuclei bright red without staining the cytoplasm significantly (try from 10 to 100 μg/mL (dilution from 1:20000~1:200). Control the staining time about 30 mins.
(2) Stain live cells：Using live cells samples, select an **GL** concentration that stains the live cell cytoplasm bright green without staining the nuclei significantly (try from 2 to 20 μg/mL (dilution from 1:20000~1:200). Control the staining time about 30 mins.
(3) In general, it is best to use the lowest dye concentration that gives sufficient signal. The reagent concentrations determined in steps (2) and (3) are optimal for the viability experiments.

2. Fluorescence microplate reader：

2.1 Select the Optical Filters for the Microplate Reader：

a. **RD** fluorescence dye for dead cell：
Exciting at 530 ± 10 nm；emission at 645 ± 20 nm.
b. **GL** fluorescence dye for live cell：
Exciting at 485 ± 10 nm；emission at 530 ± 10 nm.

2.2 Prepare the Cells for the Microplate Reader：

a. Adherent cells：Culture adherent cells in the multi-well plate. Fibroblast cells are typically grown in the wells for 2–3 days until acceptable cell densities are obtained. The cell samples are washed to remove or to dilute esterase activity generally present in serum supplemented growth media that could cause an increase in extracellular fluorescence.
b. Non-adherent cells：Wash relatively nonadherent cells (e.g., leukocytes or other suspended cells) in a test tube with 500–1000 volumes of D-PBS and sediment by centrifugation to remove serum esterase activity。
c. After the last wash, add sufficient D-PBS to at least cover the bottom of the well. In general, for flat-bottomed wells where the total capacity is 250–300 μL, add about 100 μL; for round bottomed wells where the total capacity is 150–200 μL, add about 70 μL; for conical wells where the total capacity is 100–150 μL, add about 50 μL. Small buffer volumes may be preferred to minimize dilution of cytotoxic agents and other reagents.
d. Prepare dead cells：
Before staining, a set of dead cells with different concentrations should be prepared to calculate the live cell percentage. You can kill cells by several reagents, please refer to **step 1.5a**.

2.3 Sample Preparation Example for Microplate Reader Measurements：9 mL of the

Live/Dead dual-staining working solution and 1 mL of single-staining working solution (Users can adjust the volume proportionally according to the demand)：

a. Allow the reagents to warm to room temperature (about 30 mins) and centrifuge briefly before opening.
b. Add 5 µL of the supplied **RD** stock solution to 5 mL of sterile, tissue culture–grade D-PBS, vortexing to ensure mixing thoroughly. This gives an approximately 10 µg/mL 的 **RD** solution.
c. Add 5 µL of the supplied **GL** stock solution to another 5 mL of sterile, tissue culture–grade D-PBS, vortexing to ensure mixing thoroughly. This gives an approximately 4 µg/mL 的 **GL** solution.
d. Add 0.5 mL of the **RD** solution and **GL** solution (procedure **2.3b** and **2.3c**) into two 0.5 mL of sterile, tissue culture–grade D-PBS, vortexing to ensure mixing thoroughly. This gives an 1.0 mL of 10 µg/mL **RD** working solution and 1.0 mL of 2 µg/mL **GL** working solution.
e. Mixing and vortexing the rest of 4.5 mL **RD** and **GL** solutions that prepared in procedures **2.3b** and **2.3c**. This gives a 9 mL of mixing LIVE/DEAD working solution (2 µg/mL **GL** / 10 µg/mL **RD**).

Note：Users can determine the optimal concentration of **RD** and **GL** solutions according to procedure **1.5b**.

2.4 Fluorescence Measurements Using a Microplate Reader：

a. Distribute 100 µL of cell-containing samples of **EXPERIMENTAL** buffer to each well (A and B below). Add 100 µL of Live/Dead dual-staining working solution (procedure **2.3e**) to the wells. Incubate the samples for the optimal time interval at room temperature for 30–45 minutes.
   (1) Fluorescence at 645 nm in the experimental A below cell samples, labeled with (A_{645})；
   (2) Fluorescence at 530 nm in the experimental B below cell samples, labeled with (B_{530})
b. Distribute 100 µL of cell-containing samples of **CONTROLS** buffer to each well. The samples of dead cells (C and D below)；live cells(E and F below)：
   (1) Add 100 µL of **RD** working solution (procedure **2.3d**) to the wells of C below(dead cells). Incubate the samples for 30–45 minutes at room temperature. Fluorescence at 645 nm in the C below cell samples, labeled with(C_{645})；
   (2) Add 100 µL of **GL** working solution (procedure **2.3d**) to the wells of D below(dead cells). Incubate the samples for 30–45 minutes at room temperature. Fluorescence at 645 nm in the D below cell samples, labeled with(D_{645})；
   (3) Add 100 µL of **RD** working solution (procedure **2.3d**) to the wells of E below(live cells). Incubate the samples for 30–45 minutes at room temperature. Fluorescence at 530 nm in the E below cell samples, labeled with(E_{530})；
   (4) Add 100 µL of **GL** working solution (procedure **2.3d**) to the wells of F below(live cells). Incubate the samples for 30–45 minutes at room temperature. Fluorescence at 530 nm in the F below cell samples, labeled with(F_{530})；
c. Distribute 200 µL of cell-free and/or dye-free samples of **BLANK** buffer to each well of G and H below.

(1) Fluorescence at 645 nm in the blank G below cell-free samples, labeled with (G^0_{645})；

(2) Fluorescence at 530 nm in the blank H below cell-free samples, labeled with (H^0_{530})

2.5 Interpretation of the Results：

The relative numbers of live and dead cells can be expressed in terms of percentages. The live cells emit fluorescence at about 530 nm and limited fluorescence signal at longer wavelengths. Dead cells are characterized by intense fluorescence at >600 nm and little fluorescence around 530 nm. Background fluorescence readings (G^0_{645} and H^0_{530}) may be subtracted from all values respectively prior to calculation of results.

a. Dead Cells (%)：

$$\text{Dead cells (\%)} = \frac{A_{645} - D_{645}}{C_{645} - D_{645}} X\ 100\%$$

b. Live cells (%)：

$$\text{Live cells (\%)} = \frac{B_{530} - E_{530}}{F_{530} - E_{530}} X\ 100\%$$

2.6 Determining Absolute Numbers of Live and Dead Cells Using a Microplate Reader：

The total number of cells in a sample can be counted by killing all of the cells (see procedure 1.5a), labeling with a saturating concentration of RD solution and measuring fluorescence at 645 nm. The fluorescence intensity is then linearly related to the total number of dead cells present in the sample. This may be done at the end of a set of viability experiments in order to express cell viability in terms of absolute numbers of live and dead cells：

a. Perform the cell-viability measurements (procedures 2.4-2.5).
b. Kill all of the cells in the samples, i.e. add 2–5 µL per well from a 5% saponin stock solution in distilled water.。
c. Mix by shaking the plate; wait 10 minutes (or until the signal equilibrates).
d. Read the RD fluorescence at 645 nm. The fluorescence intensity is linearly related to the number of dead cells in the sample. This value can be compared to a standard curve of numbers of dead cells vs. fluorescence intensity, generated separately by using a saturating RD concentration on known numbers of dead cells in a microplate.

2.7 Problems and solutions：

a. Determine the Optimal Dye Concentrations：

The optimal dye concentrations are likely to vary depending on the cell type. If the above method (procedure 2.3) is not suitable for your cells, please try the following methods to determine the best operating dye concentration (you can carry out either at room temperature or at a constant temperature, such as 37 °C):

(1) Stain dead cells：Using dead cells samples, select an RD concentration that stains the dead cell nuclei (try from 10 to 100 µg/mL (dilution from 1:2000~1:200)). Check the fluorescence signals at 645 nm every 10~15 mins during the staining time. In general, if the dead cell concentration is 120,000 cells per well, using 10 µg/mL RD (Dilution ratio：2000X), the fluorescence signal reaches saturation within 45 minutes of staining. Using the dead cells samples, select the HIGHTEST GL

concentration that **CAN NOT** staining the dead cell cytoplasm significantly (try from 2 to 20 μg/mL (dilution from 1:20000~1:200)). Check the fluorescence signals at 530 nm every 10~15 mins during the staining time.

(2) Stain live cells：Use the highest **GL** concentration that checked in the above step to stain the lowest concentration of live cells, so that the live cells will show obvious fluorescent signals in the reader. If there is no obvious fluorescence, try increasing the concentration of **GL** solution, or increase the concentration of live cells.

3. Flow Cytometry Protocol: Viability Assay

3.1 Allow all reagents to come to room temperature.

3.2 Make an 80-fold dilution of **GL** in DMSO working solution：(50 μg/mL, i.e., add 2 μL of **GL** to 158 μL DMSO). The working solution should be used within one day.

3.3 Prepare a 1 mL suspension of cells with 0.1 to 5 × 10^6 cells/mL for each assay. Cells may be in culture medium or buffer.

3.4 Add 2 μL of 50 μg/mL **GL** DMSO working solution and 1 μL **RD** stock solution to each mL of suspension cells solution . Mix the sample. Incubate the cells for 15–30 minutes at room temperature, protected from light.

3.5 As soon as possible after the incubation period (within 1–2 hours), analyze the stained cells by flow cytometry using 488 nm excitation and measuring green fluorescence emission for **GL** (i.e., 530/30 bandpass) and red fluorescence emission for **RD** (i.e., 610/20 bandpass). Gate on cells to exclude debris. Using single color-stained cells, perform standard compensation. The population should separate into two groups: live cells will show green fluorescence and dead cells will show red fluorescence.

Troubleshooting

1. Dye deterioration：

The live cell stain (GL, brown vial, green label) will not deteriorate under normal storage conditions. If it is deteriorated, it will produce strong fluorescence, resulting in a decrease in sensitivity.

2. For other questions, please contact our technical staff.

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