Genescence ViAlive Dual Stain Kit Manual

Introduction

The Genescence ViAlive Dual Stain Kit is a fluorescence-based assay designed to evaluate cell viability by distinguishing live and dead cells. This kit utilizes fluorescein diacetate (FDA) and propidium iodide (PI) to label viable and non-viable cells, respectively. FDA stains live cells with green fluorescence as it is converted by intracellular esterases, while PI stains dead cells by binding to DNA and emitting red fluorescence.

Kit Components and Storage

Component	Description
Green Fluorescent Dye	FDA dissolved in 1 mL acetone (store at -20°C)
Red Fluorescent Dye	PI dissolved in 1 mL PBS (store at 4°C)
Dilution Buffer	Phosphate Buffered Saline 40 mL (store at 4°C)

Prepare fresh staining solutions for each experiment, and store unused solutions as indicated to maintain kit efficacy.

Preparation of Staining Solution

Prepare the staining solution by combining the following components:

• Serum-free medium: 8 mL
• FDA Solution: 1 mL
• PI Solution: 1 mL

Ensure the solution is freshly prepared, kept protected from light, and used within 2 hours. Store on ice when not in use.

Staining Protocol

1. Fluorescent Microscopy Imaging Protocol (100 Sample for 96-well plate)

This protocol describes the process for imaging cells stained with FDA and PI under a fluorescent microscope. Green fluorescence (FDA) indicates live cells, while red fluorescence (PI) highlights dead cells.

Steps:

1. Prepare the Staining Solution:
    - Serum-free medium: 8 mL
    - FDA Solution : 1 mL
    - PI Solution : 1 mL
    - Combine the components in a sterile tube, mix thoroughly, and protect from light. Use within 2 hours.
2. Seed cells into a 96-well plate at the desired density and incubate until adherent.
3. Remove the culture medium and wash cells with PBS.
4. Add 100 µL of the staining solution to the cells, ensuring they are covered, and incubate at room temperature for 4–5 minutes in the dark.
5. Carefully remove the staining solution and wash cells with PBS to remove excess dye.
6. Add PBS to prevent cell drying.
7. Observe the cells under a fluorescence microscope:
    - **FDA (Green)**: Excitation at 488 nm, Emission at 520 nm.
    - **PI (Red)**: Excitation at 535 nm, Emission at 617 nm.
8. Capture images from multiple fields to ensure representative results.

2. Flow Cytometry Analysis Protocol (40 sample for Polystyrene tubes)

This protocol enables the quantitative assessment of live and dead cells using FDA and PI via flow cytometry.

Steps:

1. **Prepare the Staining Solution**:

    - Serum-free medium: 18 mL
    - FDA Solution : 1 mL
    - PI Solution : 1 mL
      Mix the components as described above and protect from light. Use within 2 hours.

2. Harvest cells and centrifuge to pellet them.
3. Wash the cell pellet with PBS and centrifuge again.
4. Resuspend the cell pellet in 0,5 mL of staining solution.
5. Incubate at room temperature in the dark for 4–5 minutes.
6. Wash the cells with PBS by centrifugation to remove excess dye.
7. Resuspend the cells in 500 µL PBS for analysis.
8. Analyze the cells using a flow cytometer with the following settings:

    - **FDA (Green)**: Excitation at 488 nm, Emission at 520 nm.
    - **PI (Red)**: Excitation at 535 nm, Emission at 617 nm.

9. Gate the live (FDA+) and dead (PI+) populations for quantitative analysis.

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3. Fluorescent Microplate Reader Protocol (100 sample for 96-well plate)

This protocol describes the process for high-throughput viability measurements using a fluorescent microplate reader.

Steps:

1. **Prepare the Staining Solution**:

    - Serum-free medium: 8 mL
    - FDA Solution : 1 mL
    - PI Solution : 1 mL
      Prepare as described above and use within 2 hours.

2. Seed cells into a 96-well plate at the desired density and incubate until adherent.
3. Remove the culture medium from each well and wash with PBS.
4. Add 100 µL of the staining solution to each well.
5. Incubate at room temperature in the dark for 4–5 minutes.
6. Carefully remove the staining solution and wash each well with PBS.
7. Add 100 µL PBS or serum-free medium to each well for measurement.
8. Measure fluorescence using a microplate reader:

    - **FDA (Green)**: Excitation at 488 nm, Emission at 520 nm.
    - **PI (Red)**: Excitation at 535 nm, Emission at 617 nm.

9. Subtract background fluorescence from wells without cells to calculate relative fluorescence units (RFU) for live and dead cells.