

# ELISA PRODUCT INFORMATION & MANUAL

Live-Dead Cell Staining Kit (Fluorometric) (Fluorometric) NBP2-54864

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.

Not for diagnostic or therapeutic procedures.

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#### 1. Overview

Distinguishing between live and dead cells is very important for investigation of growth control and cell death. Novus Biologicals's Live-Dead Cell Staining Kit (Fluorometric) provides the ready-to-use reagents for convenient discrimination between live and dead cells.

The kit utilizes a cell-permeable green fluorescent dye (Ex/Em = 488/518 nm), to stain live cells. Dead cells can be easily stained by propidium iodide (PI), a cell non-permeable red fluorescent dye (Ex/Em = 488/615). Stained live and dead cells can be visualized by fluorescence microscopy using a band-pass filter (detects FITC and rhodamine). The kit provides sufficient reagents for 100 stainings using 24-well plate.

## 2. Protocol Summary

Collect Cell Sample

Re-Suspend in Staining Solution

Place Sample on Glass Slide

Observe Under Fluorescence Microscope

## 3. Components and Storage

#### A. Kit Components

ltem	Quantity
Solution A (1 mM Live-Dye)	50 µl
Solution B (2.5 mg/ml PI)	50 µl
Staining Buffer	50 mL

<sup>\*</sup> Store kit at -20°C. Protect from light. Store Staining Buffer at +4°C after opening. All reagents are stable for 1 year under proper storage conditions. Please read the entire protocol before performing the assay.

#### **B.** Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescence microscope
- Glass slides and coverslips
- Orbital shaker

### 4. Assay Protocol

**1.** Prepare enough Staining Solution for your assay (0.5 ml per well in 24 well dish):

Mix 1  $\mu$ I of Solution A and 1  $\mu$ I of Solution B in 1 ml of Staining Buffer. Scale up accordingly for larger numbers of assays.

- **2.** Collect cells (1 x  $10^6$  cells) by centrifugation at 500 x g for 5 min.
- 3. Re-suspend to 0.5 ml Staining Solution
- Incubate for 15 min at 37°C.
- **5.** Place the cell suspension on a glass slide. Cover the cells with a glass coverslip.

#### Note:

For analyzing *adherent cells*, grow cells directly on a coverslip. Following incubation with the Staining Solution, invert coverslip on a glass slide and visualize cells.

**6.** Observe cells immediately under a fluorescence microscope using a band-pass filter (detects fluorescein and rhodamine).

Healthy cells stain only the cell-permeable Live-Dye, fluorescing green. Dead cells can stain both the cell-permeable Live-Dye and the cell non-permeable PI (red), the overlay of green and red appears to be yellow-red.

#### Notes:

- a) As the optimal staining conditions may vary among different cell types, we recommend that a suitable concentration of Solution A and B be determined individually.
- b) Please note that PI is suspected to be highly carcinogenic, so careful handling of the reagent is required.