

## TUNEL Apoptosis Assay Kit

Cat.: T2190

Size: 50T

### Kit Components:

| Components                      | Storage                                     | Amount          |
|---------------------------------|---|-----------------|
| Component A: 100× Tunnelyte Red | Freeze(<-15°C), Minimize light exposure     | 1 vial (25μL)   |
| Component B: Reaction Buffer    | Refrigerate(2-8°C), Minimize light exposure | 1 bottle (10mL) |
| Component C: 1000× Hoechst      | Freeze(<-15°C), Minimize light exposure     | 1 vial (50μL)   |

### Introduction:

DNA fragmentation represents a characteristic of late stage apoptosis. DNA fragmentation in apoptotic cells can be detected by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL). The TUNEL assay relies on the presence of nicks in the DNA which can be identified by TdT, an enzyme that catalyzes the addition of dUTPs that are secondarily labeled with a marker. All the existing TUNEL assays contain the highly toxic sodium cacodylate which might induces apoptosis and also decrease DNA production and DNA strands. Our Cell Meter™ TUNEL Apoptosis Assay Kit uses proprietary buffer system free of sodium cacodylate. The kit is based on the incorporation of our unique proprietary fluorescent dye into the DNA fragments that form during apoptosis. The assay is optimized for the direct detection of apoptosis in either detached or attached cells without using antibody. The kit provides all the essential components with an optimized assay protocol. It is suitable for fluorescence microplate reader, fluorescence microscope, or flow cytometer.

### Protocol summary:

- 1、Prepare cells with test compounds
- 2、Incubate with TUNEL working solution for 30 min to 1 hour at 37°C
- 3、Wash the cells
- 4、Fix cells with 4% formaldehyde (optional)
- 5、Read fluorescence intensity at Ex/Em=550/590-650nm (Cutoff=570nm), fluorescence microscope with TRITC filter or flow cytometer with FL3 channel

**Note:** Thaw all the components at room temperature before starting the experiment.

### Key parameters:

Instrument: Fluorescence microscope

Excitation: TRITC filter

Emission: TRITC filter

Recommended plate: Black wall/clear bottom

Instrument: Flow cytometer

Excitation: 488nm laser

Emission: 660/20nm filter

Instrument specification(s): PE-Cy5 channel

Instrument: Fluorescence microplate reader

Excitation: 550nm

Emission: 590-650nm

Cutoff: 570nm

Recommended plate: Solid black

### ***PREPARATION OF WORKING SOLUTION:***

Add 0.5μL of 100× Tunnelyte Red (Component A) into 50μL of Reaction Buffer (Component B) to make a total volume of 50.5μL of TUNEL working solution. Protect from light.

**Note:** Each cell line should be evaluated on an individual basis to determine the optimal cell density.

### ***SAMPLE EXPERIMENTAL PROTOCOL:***

Culture cells to an optimal density for apoptosis induction according to your specific protocol. We recommend about 30,000 to 50,000 cells/well for adherent cells grown in a 96-well microplate culture, or about  $1$  to  $2 \times 10^6$  cells/mL for non-adherent cells. At the same time, culture a non-induced negative control cell population at the same density as the induced population for every labeling condition.

**Note:** We treated HeLa cells with 100nM - 1μM staurosporine for 4 hours to induce cell apoptosis. See Figure 1 for details.

### ***Stain and Fixation:***

- 1、 Remove cell media.
- 2、 Add 50μL of TUNEL working solution to each sample.
- 3、 Incubate at 37°C for 30-60 minutes.
- 4、 Remove TUNEL working solution, and wash the cells 1-2 times with 200μL/well of PBS.
- 5、 Add 100μL Reaction buffer (Component B) to each sample.
- 6 、 Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em= 550/590-650nm (Cutoff = 570nm), fluorescence microscope with TRITC filter or flow cytometer with FL3 channel

- 7、 **Optional:** Remove the reaction buffer from Step 5, and add 100 μL/well/96-well plate of 4% formaldehyde fixative buffer (not supplied) to each well.

**Note:** For non-adherent cells, add desired amount (such as  $2 \times 10^6$  cells/mL) of 4% formaldehyde fixative buffer.

- 8、 Incubate plates for 20 to 30 minutes at room temperature.
- 9、 Remove fixative.

- 10、 Wash the cells with PBS 2-3 times, and replace with 100 $\mu$ L PBS/well/96-well plate.
- 11、 Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 550/590-650nm (Cutoff = 570nm), fluorescence microscope with TRITC filter or flow cytometer with FL3 channel
- 12、 **Optional:** Stain the nucleus with 1 $\times$  Hoechst (Component C) at Ex/Em = 350/460nm for image analysis

#### Data Analysis:

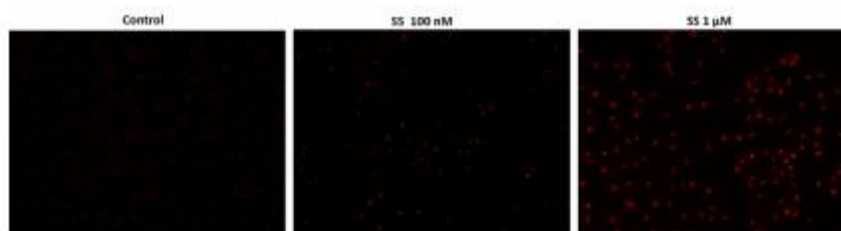


Figure 1. Fluorescence images of TUNEL reaction in HeLa cells with the treatment of 100nM or 1 $\mu$ M staurosporine (SS) for 4 hours as compare to untreated control. Cells were incubated with TUNEL working solution for 1 hour at 37°C. The red fluorescence signal was analyzed using fluorescence microscope with a TRITC filter set. Fluorescently labeled DNA strand breaks shows intense fluorescent staining in SS treated cells.