Cell Meter™ TUNEL Apoptosis Assay Kit

Red Fluorescence

Ordering Information	*Storage*	*Instrument Platform*
Product Number: 22844 (50 assays)	Keep in freezer Avoid light	Fluorescence microscope, flow cytometer and fluorescence microplate reader

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 incorporation of a red fluorescence dye modified deoxyuridine 5'-triphosphates at the 3' OH ends of 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. Its signal can be detected with a TRITC filter set or at Ex/Em = 550 nm/590-650 nm.

Kit Key Features

No Sodium Cacodylate:	No special requirements for waste treatment.
Convenient:	All essential assay components are included, no antibody is needed.
Enhanced value:	Less expensive than the sum of individual components.

Kit Components

Components	Amount
Component A: 100X Tunnelyte™ Red	1 vial (25 µL)
Component B: Reaction Buffer	1 bottle (5 mL)
Component C: 1000X Hoechst	1 vial (50 µL)

Assay Protocol:

Brief Summary

Prepare cells with test compounds → Fix cells with 4% formaldehyde → Incubate with reaction mixture for 1 hour at 37°C → Wash and analyze the cells at Ex/Em = 550 nm/590-650 nm

Note: Thaw Components C at room temperature, keep Components A and B on ice before use.

1. 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 x 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 100 nM-1µM staurosporine for 4 hours to induce cell apoptosis. See Figure 1 for details.
2. Fixation and Permeabilization
   a. Remove cell media.
   b. Add 100 µL/well/96-well plate of 4% formaldehyde fixative buffer (not supplied) to each well.

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

c. Incubate plates for 20 to 30 minutes at room temperature.

d. Remove fixative.

Optional: add 100 µL/well/96-well plate of the permeabilization reagent (0.2% Triton X-100 in PBS, not supplied) after the fixation if needed, and incubate the plate for 10 minutes at room temperature.

e. Wash the cells with PBS 2-3 times.

Optional: You may also prepare a positive control for TUNEL reaction using DNAase I by digesting cells with DNAase I for 30 min at room temperature before proceed to TUNEL reaction (Step 3)

3. TUNEL reaction

a. Prepare reaction mixture just before use based on the number of samples to be assayed:

Reaction Components	**Volume Per Well**
100X Tunnelyte™ Red (Component A)	0.5 µL
Reaction Buffer (Component B)	50 µL
Total volume	50.5 µL

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

b. Add 50 µL of the reaction mixture (from Step 3.1) to each sample and incubate at 37ºC for 60 minutes.

c. Remove the reaction mixture, and wash the cells 3-5 times with 200 µL/well of PBS.

4. Monitor the fluorescence intensity by fluorescence microscope, flow cytometer, or fluorescence microplate reader at Ex/Em = 550/590-650 nm.

5. Optional: Stain the nucleus with 1X Hoechst (Component C, Ex/Em = 350/460 nm) for image analysis.

Data Analysis:

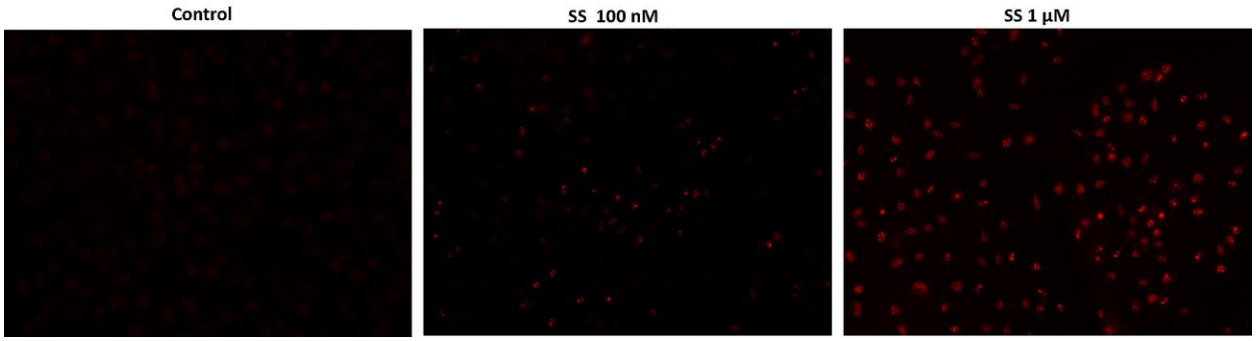

Figure 1. Fluorescence images of TUNEL reaction in HeLa cells with the treatment of 100 nM or 1 µM staurosporin (SS) for 4 hours as compare to untreated control. Cells were incubated with reaction mixture for 1 hour at 37ºC. The red fluorescence signal was analyzed using fluorescence microscope with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