Cell Meter™ TUNEL Apoptosis Assay Kit *Red Fluorescence*

Catalog number: 22844
Unit size: 50 Tests

Component	Storage	Amount
Component A: 100X Tunnelyte™ Red	Freeze (<-15 °C), Minimize light exposure	1 Vial (25 µL)
Component B: Reaction Buffer	Refrigerate (2-8 °C), Minimize light exposure	1 Bottle (10 mL)
Component C: 1000X Hoechst	Freeze (<-15 °C), Minimize light exposure	1 Vial (50 µL)

OVERVIEW

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.

AT A GLANCE

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 = 550/590-650 nm (Cutoff = 570 nm), fluorescence microscope with TRITC filter or flow cytometer with FL3 channel

Important 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:	488 nm laser
Emission:	660/20 nm filter
Instrument specification(s):	PE-Cy5 channel
Instrument:	Fluorescence microplate reader
Excitation:	550 nm
Emission:	590 - 650 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF WORKING SOLUTION

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

Note

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

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

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 - 1 µM staurosporine for 4 hours to induce cell apoptosis. See Figure 1 for details.

Stain and Fixation:

1. Remove cell media.
2. Add 50 µL 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 200 µL/well of PBS.
5. Add 100 uL Reaction buffer (Component B) to each sample.
6. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 550/590-650 nm (Cutoff = 570 nm), 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 2X10^6 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 µL PBS/well/96-well plate.
11. Monitor the fluorescence intensity with a fluorescence microplate reader at Ex/Em = 550/590-650 nm (Cutoff = 570 nm), fluorescence microscope with TRITC filter or flow cytometer with FL3 channel
12. **Optional:** Stain the nucleus with 1X Hoechst (Component C) at Ex/Em = 350/460 nm for image analysis

EXAMPLE DATA ANALYSIS AND FIGURES

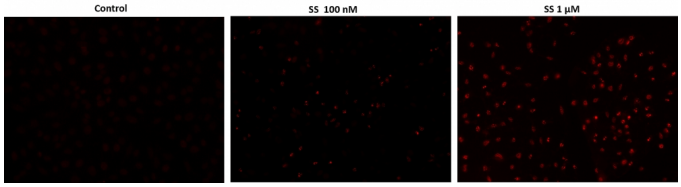

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Figure 1. Fluorescence images of TUNEL reaction in HeLa cells with the treatment of 100 nM or 1 µM staurosporine (SS) for 4 hours as compare to untreated control. Cells were incubated with TUNEL working solution for 1 hour at 37ºC. The red fluorescence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

DISCLAIMER

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