

**Cell Meter™ Live Cell TUNEL Apoptosis Assay
Kit *Red Fluorescence***

 Catalog number: 22844
Unit size: 50 Tests

Component	Storage	Amount (Cat No. 22844)
Component A: 100X Tunnelyte™ Red	Freeze (< -15 °C), Minimize light exposure	1 Vial (25 µL)
Component B: Reaction Buffer	Freeze (< -15 °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 using 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
Flow cytometer

Emission	660/20 nm filter
Excitation	488 nm laser
Instrument specification(s)	PE-Cy5 channel

Fluorescence microscope

Emission	TRITC filter
Excitation	TRITC filter
Recommended plate	Black wall/clear bottom

CELL PREPARATION

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 10⁶ 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.

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

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.

SAMPLE EXPERIMENTAL PROTOCOL
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 µL Reaction buffer (Component B) to each sample.
6. Monitor the fluorescence intensity using a 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 2X10⁶ 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 using a 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

Placeholder for image details

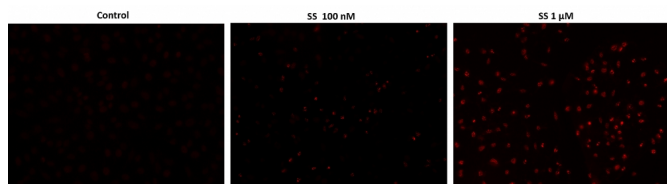


Figure 1. Fluorescence images of TUNEL reaction in HeLa cells with the treatment of 100 nM or 1 μ M staurosporine (SS) for 4 hours as compare to untreated control. Cells were incubated with TUNEL working solution for 1 hour at 37°C. The red fluorescence signal was analyzed using fluorescence microscope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

DISCLAIMER

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