

**Cell Meter™ Live Cell TUNEL Apoptosis Assay Kit \*Green Fluorescence\***

Catalog number: 22849

Unit size: 50 Tests

Component	Storage	Amount (Cat No. 22849)
Component A: 100X Tunnelyte™ Green	Freeze (< -15 °C), Minimize light exposure	Vial (25 µL)
Component B: Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	Bottle (10 mL)
Component C: 1000X Hoechst	Freeze (< -15 °C), Minimize light exposure	Vial (50 µL)

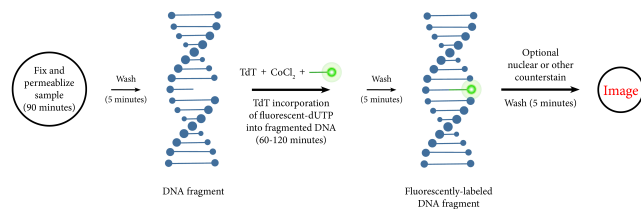
**OVERVIEW**

TUNEL assays detect DNA fragmentation in live and fixed cells, which often occur in late-stage apoptosis. Internucleosomal DNA fragmentation caused by activated endonucleases is universally regarded as the biochemical hallmark of apoptosis. Cells containing these DNA strand breaks can be identified with the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. This well-established *in situ* staining method relies on the enzyme terminal deoxynucleotidyl transferase (TdT) to catalyze the incorporation of modified dUTPs to the 3'-hydroxyl termini of fragmented DNA. Depending upon the choice of modified dUTP - BrdUTP, biotin-dUTP, or a fluorescein-dUTP - apoptotic cells can be identified and measured using various detection strategies and systems, including fluorescence microscopy, flow cytometry, and fluorescence-based microplate studies.

AAT Bioquest offers several fluorimetric TUNEL assays optimized for *in situ* apoptosis detection in live cells or fixed cells and tissue samples. These products can be combined with other cell-based viability and apoptosis assays to provide a comprehensive assessment of cell health, assess toxicity and safety of drug candidates, or analyze cancer and disease-related cellular changes.

**Principles and mechanism**

During the later stages of apoptosis, caspase-activated endonucleases cleave genomic DNA into oligonucleosomal fragments (~180-200 base pairs long). Using the TUNEL assay, the exposed 3'-OH termini of these breaks can be marked with modified dUTPs for subsequent visualization and quantification of apoptotic cells *in situ*. This is generally done either directly using dye-modified dUTP or indirectly using BrdUTP and antibody conjugates against BrdUTP. Regardless of the labeling strategy, both require the enzyme terminal deoxynucleotidyl transferase (TdT) to catalyze the incorporation of the modified dUTPs to the 3'-OH termini.



Detection of DNA fragments by this assay requires sample fixation using a crosslinking reagent such as 4% paraformaldehyde (avoid ethanol-based fixatives as it hinders the extraction of small DNA fragments) and sample permeabilization. Both steps are critical to the TUNEL assay as it facilitates the entry of exogenous TdT and antibody conjugates against BrdUTP. Keep in mind that several variables influence the staining kinetics of the TUNEL assay. Some of these variables, including reagent concentration, fixation of the sample, and accessibility of DNA strand breaks, may vary between tissue or cell sample types. Standardizing the TUNEL assay using samples with positive and negative apoptosis controls can alleviate these potential influences and reduce false positives or negative results.

**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. Monitor the fluorescence intensity in a fluorescence microscope with FITC filter or flow cytometer with FITC channel.

**Important Note**

Thaw all the components at room temperature before starting the experiment.

**KEY PARAMETERS**
**Fluorescence microscope**

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

**Flow cytometer**

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

**CELL PREPARATION**

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™ Green (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**

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<sup>6</sup> 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.

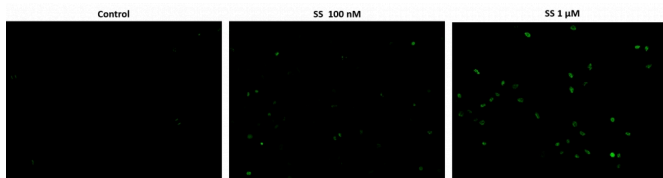
**Note:** We treated HeLa cells with 100 nM - 1  $\mu$ M staurosporine for 4 hours to induce cell apoptosis. See Figure 1 for details.

have any questions.

#### Stain and Fixation:

1. Remove cell media.
2. Add 50  $\mu$ L 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  $\mu$ L/well of PBS.
5. Add 100  $\mu$ L Reaction buffer (Component B) to each sample.
6. Monitor the fluorescence intensity with a fluorescence microscope with FITC filter set or a flow cytometer with FITC channel.
7. **Optional:** Remove the reaction buffer from Step 5, and add 100  $\mu$ L/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<sup>6</sup> 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 microscope with FITC filter set or a flow cytometer with FITC 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



**Figure 1.** Fluorescence images of TUNEL reaction in HeLa cells with the treatment of 100 nM 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 green fluorescence signal was analyzed using fluorescence microscope with a FITC filter set. Fluorescently labeled DNA strand breaks shows intense fluorescent staining in SS treated cells.

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