# M5 TUNEL 细胞凋亡检测试剂盒使用说明书

| 产品 | 单位 | 货号 |
|---|---|---|
| M5 TUNEL 细胞凋亡检测试剂盒 | 10T | MF276-T |
| M5 TUNEL 细胞凋亡检测试剂盒 | 50T | MF276-01 |

## 【储存条件】

-20℃保存。

## 【产品组分】

| | MF276-T | MF276-01 |
|---|---|---|
| 100x TF3-dUTP | 5 uL | 25 uL |
| Reaction Buffer | 2 mL | 10 mL |
| 1000x Hoechst | 10 uL | 50 uL |

## 【产品简介】

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 fluorescence dye TF3 modified deoxyuridine 5'-triphosphates (TF3-dUTP) 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 easily detected at Ex/Em = 550nm/590 nm.

## 【操作步骤】

This protocol only provides a guideline, and should be modified according to your specific needs.

**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 2x$10^6$ 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. Here are a few examples for inducing apoptosis in suspension culture:

1) Treat Jurkat cells with 2 μg/ml camptothecin for 3 hours.

2) Treat Jurkat cells with 1 μM staurosporine for 3 hours.

3) Treat HL-60 cells with 4 μg/ml camptothecin for 4 hours.

4) Treat HL-60 cells with 1 μM staurosporine for 4 hours.

2. Fixation and Permeabilization

2.1 Remove cell media.

2.2 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 x 106 cells/mL) of 4% formaldehyde fixative buffer.**

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

2.4 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.

2.5 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

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

| Reaction Components | Volume Per Well |
|---|---|
| 100X TF3-dUTP | 0.5 uL |
| Reaction Buffer | 50 uL |
| Total volume | 50.5 uL |

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

3.2 Add 50 uL of the reaction mixture (from Step 3.1) to each well or tube and incubate at 37˚C for 60minutes.

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

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

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

**【数据分析】**

1. 96-Well Fluorescence Plate Reader Sample Data:

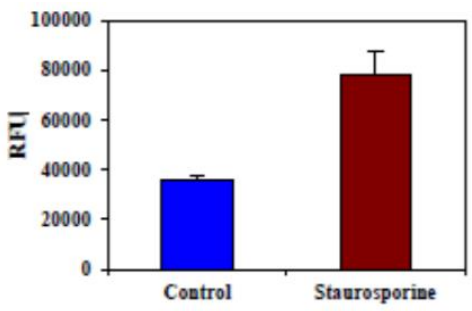
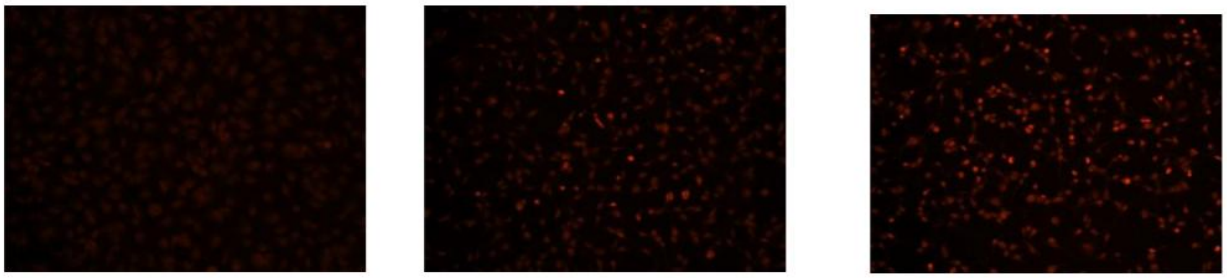



Figure 1. Apoptosis analysis in Hela cells using TUNEL Apoptosis Assay Kit. Hela cells at 30,000 cells/100µL/well were treated with 1 µM staurosporine for 4 h (Red) while un-induced cells were used as a control (Blue). Cells were incubated with reaction mixture for 1 hour at 37˚C. The Fluorescence was measured at Ex/Em = 550/590 nm (cut off at 570 nm) with a Flex Station microplate reader using bottom read mode.

2. Fluorescence Microscope Sample Data:



A.Control B. 2 hours C. 4 hours

Figure 2. The Fluorescence imagining indicated the increase in TUNEL reaction with the addition of 1 μM staurosporin for 2h (B) or 4h (C) compare to control (A) in Hela cells. Cells were incubated with reaction mixture for 1 hour at 37℃. The Fluorescence intensity of the cells (30,000 cells/ 100 μL per well) was analyzed under a fluorescence microscope with a TRITC channel. DNA strand breaks are shown as more intense fluorescent staining spots in cells treated with staurosporin.