Cell Meter™ Fixed Cell and Tissue TUNEL Apoptosis Assay Kit *Red Fluorescence*

Catalog number: 22853
Unit size: 25 Tests

Component	Storage	Amount
Component A: TdT enzyme	Freeze (< -15 °C), Minimize light exposure	1 vial (12.5 µL)
Component B: Tetramethylrhodamine-dUTP	Freeze (< -15 °C), Minimize light exposure	1 vial (12.5 µL)
Component C: CoCl2 solution	Freeze (< -15 °C), Minimize light exposure	1 vial (125 µL)
Component D: TdT Reaction Buffer	Freeze (< -15 °C), Minimize light exposure	1 vial (1.2 mL)

OVERVIEW

Cell Meter™ TUNEL apoptosis assay kit provides a robust tool for conveniently detecting apoptosis caused by DNA fragmentation. The assay is non-radioactive and rapid. The TUNEL assay uses the terminal deoxynucleotidyl transferase (TdT) to catalyze the incorporation of TMR-dUTP at the free 3'-hydroxyl ends of the fragmented DNAs. The resulted TMR-labeled DNAs are analyzed by fluorescence microscopy (Cy3 or TRITC filter set). Its red emission can be conveniently multiplexed with GFP labelled targets. Direct incorporation of fluorescent TMR-labeled nucleotides significantly reduces the number of test steps. The kit is optimized to detect apoptosis in fixed cells and formalin-fixed, paraffin-embedded tissue sections.

AT A GLANCE

Protocol summary

1. Treat samples as desired
2. Fix cells with 4% formaldehyde solution for 30 minutes on ice
3. Permeabilize cells with 70% ice-cold ethanol for 60 minutes on ice
4. Add TdT staining solution to samples and incubate for 60 minutes at 37 °C
5. Monitor the fluorescence intensity using fluorescence microscopy with Cy3 filter set

KEY PARAMETERS

Flow cytometer

Excitation	488 nm laser
Emission	575/26 nm filter
Instrument specification(s)	PE channel

Fluorescence microscope

Excitation	Cy3 filter set
Emission	Cy3 filter set
Recommended plate	Black wall/clear bottom

PREPARATION OF WORKING SOLUTION

TdT staining solution
For one test, Mix the following to make total volume of 51 µL;
45 µL TdT Reaction Buffer (Component D)
5 µL $CoCl_2$ (Component C)
0.5 µL TF5-dUTP (Component B)
0.5 µL TdT enzyme (Component A).

Note TdT staining solution should be used promptly.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline and should be optimized according to the needs.

1. Treat your samples as desired.
2. Wash the cells with buffer of your choice such as PBS containing Ca^{+2} and Mg^{+2}.
3. Fix the cells by adding 100 µL of 4% paraformaldehyde in PBS and incubate the samples for 30 minutes on ice.
4. Remove fixation solution and wash cells with PBS.
5. Add 100 µL of 70% of ice cold ethanol to cells and incubate the samples for 60 minutes on ice.

   Note Cells can be stored at -20 °C at this step for several days before use.
6. Remove alcohol and wash cells with PBS.

   Note For a positive control, incubate fixed cells with 2-5 µg/mL of DNAse in PBS containing Ca^{+2} and Mg^{+2} for 60 minutes at 37 °C. Remove the DNAse and wash cells thoroughly and continue with the rest of the protocol.
7. Add 50 µL of TdT staining solution to the cells and incubate for 60 to 120 minutes at 37 °C.
8. Remove TdT working solution and wash cells with PBS.
9. Resuspend the cells in PBS and monitor the fluorescence intensity with flow cytometer using 575/26 nm filter (PE channel) or fluorescence microscope with Cy3 filter set.

Protocol for tissue staining
The following protocol can be used as a guideline and should be optimized according to the needs.
Deparaffinization and rehydration protocol

1. Deparaffinize tissue sections (attached to the microscopic slides) by immersing slides in fresh xylene in a Coplin jar for 5 minutes at room temperature. Repeat one more time. (Total 2 washes)
2. Wash the samples by immersing the slides in 100% ethanol for 5 minutes at room temperature in a Coplin jar.
3. Rehydrate the samples by immersing the slides through various concentrations of alcohol subsequently (100, 95, 85, 70, 50%) for 5 minutes each at room temperature.
4. Wash the samples by immersing the slides in 0.85% NaCl for 5 minutes at room temperature.
5. Wash the samples by immersing the slides in PBS for 5 minutes at room temperature. Repeat one more wash. (Total 2 washes)

Fixation protocol

1. Fix the tissue sections by immersing slides in 4% paraformaldehyde solution in PBS for 15-20 minutes at room temperature.

2. Wash the samples by immersing the slides in PBS for 5 minutes at room temperature. Repeat one more wash. (Total 2 washes)

3. Remove the liquid and place the slides on a flat surface. Treat tissue sections with 100 µL of 20 µg/mL Proteinase K solution. Add enough to cover the entire tissue surface. Incubate slides for 10 minutes at room temperature.

4. Wash the samples by immersing the slides in PBS for 5 minutes at room temperature.

5. Fix the tissue sections by immersing slides in 4% paraformaldehyde solution in PBS for 15-20 minutes at room temperature.

6. Wash the samples by immersing the slides in PBS for 5 minutes at room temperature. Repeat one more wash. (Total 2 washes)

Staining protocol

1. Optional: For a positive control, incubate fixed samples with 2-5 µg/mL of DNAse in PBS containing Ca^{+2} and Mg^{+2} for 60 minutes at 37 °C. Remove the DNAse and wash cells thoroughly with PBS and continue with the rest of the protocol.

2. Add 50 µL of TdT staining solution to the samples and incubate for 60 to 120 minutes at 37 °C.

3. Remove TdT working solution and wash samples with PBS.

4. Add mounting medium with DAPI (AAT Bioquest Cat# 20005) and monitor the fluorescence intensity fluorescence microscope with Cy3 filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

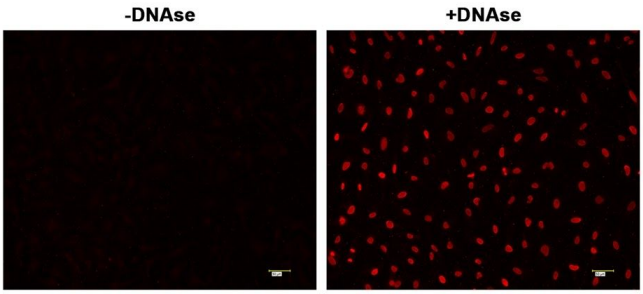

Figure 1. Fluorescence images of TUNEL assay with HeLa cells.
HeLa cells were fixed and treated with or without DNAse for 60 mins at 37 °C. The cells were then stained with Cell Meter™ Fixed Cell and Tissue TUNEL Apoptosis Assay Kit. DNA strand breaks showed intense fluorescent staining in DNAse treated cells. The signal was acquired with fluorescence microscope using a Cy3 filter set.

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