TUNEL Apoptosis Assay (TUNEL)

Cat. No. 8088, 50 tests

Introduction

The ScienCell™ TUNEL Apoptosis Assay is used for detection of apoptosis (programmed cell death) in individual cells based on the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end-labeling (TUNEL) technology. In brief, the 3'-OH end of the DNA strand breaks in apoptotic cells is labeled with biotinylated nucleotides using the enzyme TdT. Streptavidin conjugated horseradish peroxidase (HRP) is then bound to the biotinylated nucleotides, and visualized using the peroxidase substrate, 3,3'-diaminobenzidine tetrachloride (DAB). The nuclei of apoptotic cells should be observed dark blue to bluish black under light microscope.

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8088a	1	Equilibrium Buffer	5 ml	-20ºC
8088b	1	TdT Solution	250 µl	-20ºC
8088c	1	Biotin-dUTP Solution	2.5 ml	-20ºC
8088d	1	Stock Streptavidin-HRP (100×)	50 µl	-20ºC
8088e	1	DAB Substrate	Tablets	-20ºC
8088f	1	DNase I	1 ml	-20ºC

Materials to be Supplied by the User

PBS
Paraformaldehyde
Triton X-100
Methanol
H_2O_2
Hematoxylin (optional)

Quality Control

The ScienCell™ TUNEL Apoptosis Assay is applied to Human Astrocytes (HAs) treated with and without DNase I, which serve as our positive and negative controls, respectively. Dark nucleus can only be observed in cells treated with DNase I (Figure 1).

The ScienCell™ TUNEL Apoptosis Assay is also applied to Rat Cortical Neurons cultured with ScienCell™ Neuronal Medium for 3 days, and apoptotic cells can be identified with dark nucleus (Figure 2).

Procedures (96-well plate)

A. Cell culture

1. Seed cells in 96-well culture plate in culture medium with or without test compounds. Culture the cells in a CO_2 humidified incubator at 37°C for the desired period of time.

B. Pretreatment of cells

1. Rinse cells three times with PBS, and fix cells by incubating with freshly prepared 3.7% paraformaldehyde in PBS for 10 minutes at room temperature.
2. Wash cells three times with PBS, for 2 minutes each time.
3. Permeabilize cells by incubating with 0.2% Triton X-100 in PBS for 15 minutes at room temperature.
4. Wash cells three times with PBS, for 2 minutes each time.

C. Setup of positive controls (optional)

1. Incubate positive control samples with DNase I (100 µl per well) for 10 minutes at room temperature to induce cleavage of genomic DNA.

D. TUNEL staining of cells

1. Pre incubate cells with 100 µl/well of Equilibrium Buffer for 10 minutes at room temperature.
2. Prepare TUNEL reaction mixture just before use based on the number of samples to be assessed. For each well of 96-well plate, mix 5 µl of TdT Solution with 45 µl of Biotin-dUTP Solution.
3. Incubate cells in TUNEL reaction mixture (50 µl per well) for 60 minutes at 37ºC, protected from light.
4. Wash cells three times with PBS, for 2 minutes each time.
5. Block the endogeneous peroxidase activity by incubating cells with 100 µl per well of 0.3% H_2O_2 in methanol for 10 minutes at room temperature, protected from light.
6. Wash cells three times with PBS, for 2 minutes each time.
7. Prepare working streptavidin-HRP by diluting Stock Streptavidin-HRP 100× with PBS. Add 100 µl of working streptavidin-HRP to each well of 96-well plate, and incubate for 30 minutes at 37ºC, protected from light.
8. Wash cells three times with PBS, for 2 minutes each time.
9. Prepare working DAB substrate by adding 5ml of DI H_2O to the vial of DAB Substrate (Cat #8088e). For best results use the solution immediately. Add 100 µl of working DAB substrate to each well of 96-well plate, and incubate for 1-5 minutes at room temperature in the dark, or until a blue background develop.
10. Wash cells three times with PBS, for 2 minutes each time.
11. Counter stain cells with hematoxylin if needed.
12. Observe staining with a light microscope.

A. **B.**

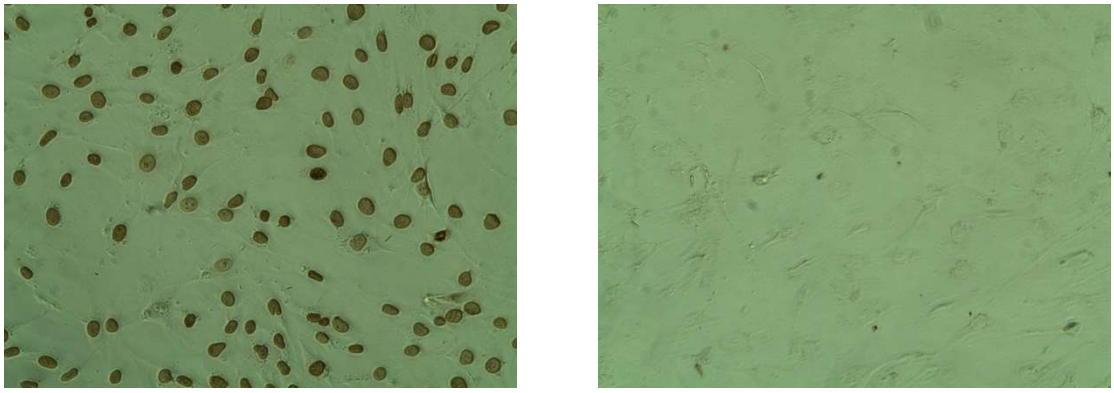

Figure 1. The ScienCell™ TUNEL Apoptosis Assay is applied to human astrocytes treated with (A) and without (B) DNase I, and data shows that dark nuclei can only be observed in those cells treated with DNase I.

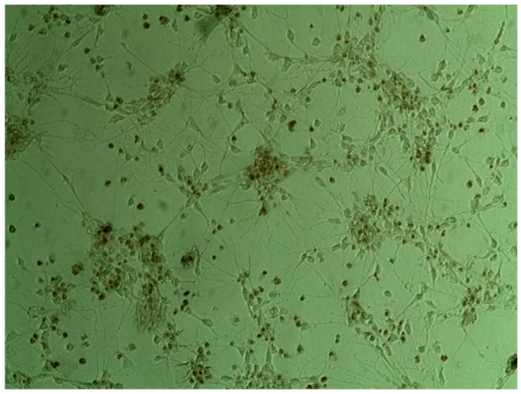

Figure 2. The ScienCell™ TUNEL Apoptosis Assay is applied to rat cortical neurons cultured with ScienCell™ Neuronal Medium for 3 days, and apoptotic cells can be identified with dark nucleus.