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One Step TUNEL Apoptosis Detection Kit (Cyanine 3)

1 Contents

Components	HY-K1079-20T	HY-K1079-50T
TdT Enzyme	100 μL	250 μL
Cy3-dUTP Labeling Mix	900 μL	2 × 1.2 mL
TdT Dilution Buffer	300 μL	1 mL

2 Introduction

MCE One Step TUNEL Apoptosis Detection Kit (Cyanine 3) provides a rapid and convenient method to detect cell apoptosis. When cells undergo apoptosis, specific DNA endonucleases will be activated, cutting the genomic DNA between the nucleosomes. The DNA of apoptotic cells is cleaved into multimers of 180-200 bp fragments. The exposed 3'-OH of the broken DNA can be catalyzed by Terminal Deoxynucleotidyl Transferase (TdT) with fluorescein labeled dUTP, which can be detected with fluorescence microscope or flow cytometer. The maximum excitation wavelength and emission wavelength of Cy3 (Cyanine 3) were 550 nm and 570 nm respectively.

3 General Protocol

Recommended Buffer

Fixation solution	4% Paraformaldehyde in PBS, pH 7.4, freshly prepared
Permeabilisation solution	0.3% Triton X-100 in PBS, pH 7.4, freshly prepared
Proteinase K solution	20 μg/mL Proteinase K (HY-108717) in 10 mM Tris-HCl, pH 7.4, no DNase

Sample Preparation

For adherent cells

a. Discard the medium and wash twice with PBS, 5 minutes each time.

b. Incubate cells in Fixation solution for 30 minutes at 4°C.

c. Wash twice with PBS, 5 minutes each time.

d. Resuspend cells in Permeabilisation solution for 5 minutes at room temperature.

e. Wash twice with PBS, 5 minutes each time.

For suspension cells

a. Collect 2 × 10^6 cells by centrifugation. Wash twice with PBS, 5 minutes each time.

b. Incubate cells in Fixation solution for 30 minutes at 4°C.

Note: It is recommended to incubate on a shaker during fixation to avoid extensive clumping of cells.

c. Wash twice with PBS, 5 minutes each time.

d. Resuspend cells in Permeabilisation solution for 5 minutes at room temperature.

e. Wash twice with PBS, 5 minutes each time.

For paraffin-embedded tissue

a. Dewax the tissue twice in xylene, 5-10 minutes each time.

b. Rehydrate the tissue through a graded series of ethanol (100%、90%、80%、70%).

c. Wash twice with PBS, 5 minutes each time.

d. Incubate tissue section for 15-30 minutes at 20-37°C with Proteinase K solution.

e. Wash twice with PBS, 5 minutes each time.

For frozen tissue

a. Incubate cells in Fixation solution for 30-60 minutes at 4°C.

b. Wash twice with PBS, 10 minutes each time.

c. Resuspend cells in Permeabilisation solution for 5 minutes at room temperature.

d. Wash twice with PBS, 5 minutes each time.

Note: All processed samples should be placed in the wet box to avoid dry.

Preparation of TUNEL working solution

Prepare appropriate TUNEL working solution as below.

	One sample	Five samples	Ten samples
TdT Enzyme	5 μL	25 μL	50 μL
Cy3-dUTP Labeling Mix	45 μL	225 μL	450 μL
Total volume	50 μL	250 μL	500 μL

Note: The TUNEL working solution should be prepared immediately before use and should not be frozen. Keep TUNEL working solution away from light.

Labeling and Detection

For adherent cells or tissues

Add 50 μL of TUNEL working solution and incubate at 37°C for 60 minutes in the dark. Wash three times with PBS. Add one drop of Antifade Mounting Medium (HY-K1042) to the sample and then detect the fluorescence by fluorescence microscope.

Note: a. To ensure a homogeneous spread of TUNEL working solution across cell monolayer and to avoid evaporative loss, samples should be covered with parafilm or coverslip during incubation.

b. Add 100 μL of TUNEL working solution per well for 6-well plate.

c. Dilute the TdT Enzyme 2-5 times with TdT Dilution Buffer when the TUNEL assay is too strong.

For suspension cells

Add 50 μL of TUNEL working solution and incubate at 37°C for 60 minutes in the dark. Wash three times with PBS. Resuspend the cells with 250-500 μL of PBS and then detect the fluorescence by flow cytometer or fluorescence microscope.

Note: Dilute the TdT Enzyme 2-5 times with TdT Dilution Buffer when the TUNEL assay is too strong.

4 Storage

-20°C, 1 year

Protect from light

5 Precautions

1. Detect the fluorescence as soon as possible to avoid fluorescence quenching.
2. The TUNEL working solution should be prepared immediately before use and should not be frozen. Keep TUNEL working solution away from light.
3. All processed samples should be placed in the wet box to avoid dry.
4. Dilute the TdT Enzyme 2-5 times with TdT Dilution Buffer when the TUNEL assay is too strong.
5. It is recommended to incubate on a shaker during fixation to avoid extensive clumping of cells.
6. This product is for R&D use only, not for drug, household, or other uses.
7. For your safety and health, please wear a lab coat and disposable gloves to operate.