

VitroView™ TUNEL Apoptosis Kit (50 assays)
Cat. No. VB-4005

Introduction

DNA fragmentation represents a characteristic hallmark of apoptosis. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is designed to detect apoptotic cells that undergo extensive DNA degradation during the late stages of apoptosis. The method is based on the ability of Terminal deoxynucleotidyl transferase (TdT) to label blunt ends of double-stranded DNA breaks independent of a template.

Kit Components

1. Proteinase K stock solution (20×) 0.25 mL
2. TdT equilibration buffer 1 mL
3. TdT enzyme (20 U/μL) 50 μL
4. Biotinylated dUTP 20 μL
5. RTU Streptavidin-HRP 5 mL
6. DAB stock solution (40×) 0.5 mL
7. Stable H₂O₂ solution (40×) 0.5 mL
8. DAB enhancer buffer 15 mL

Storage

RTU Streptavidin-HRP: Store at 4 °C.
Others: Store at -20 °C.

Protocol

1. Preparation of Slides

A. Frozen Sections

- 1) Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store the frozen tissue block at -80°C until ready for sectioning.
- 2) Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
- 3) Section the frozen tissue block into a desired thickness (typically 5-10 μm) using the cryotome.
- 4) Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost)
- 5) Sections can be stored in a sealed slide box at -80°C for later use.
- 6) Before staining, warm slides at room temperature for 30 minutes and fix slides by 2% neutralized formalin for 30 minutes. Rinse slides with PBS then transfer to a Coplin jar

containing ice-cold 70% ethanol for 1 hour. Slides may be stored overnight in 70% ethanol at 4°C.

7) Wash in PBS.

8) Follow procedure for pretreatment as required with TdT reaction buffer.

B. Paraffin Sections

1) Deparaffinize sections in xylene, 3×5 minutes.

2) Hydrate with 100% ethanol, 2×2 minutes.

3) Hydrate with 95% ethanol, 2×2 minutes.

4) Rinse in distilled water.

5) Prepare 1mL of 1×proteinase K working solution by mixing 50μL of 20×proteinase K solution with 950 μL of TE(Tris-EDTA) buffer.

6) Prepare carefully blot away excess water and pipette 75-100 μL of 1×proteinase K solutions to cover sections. Incubate 15 minutes at room temperature.

7) Following proteinase K treatment, wash slides 3×5 minutes with ddH₂O.

8) Inactivate endogenous peroxidases by covering sections with 2% hydrogen peroxide for 5 minutes at room temperature. Wash slides 3×5 minutes with ddH₂O.

9) Follow procedure for pretreatment as required with TdT reaction buffer.

C. Adherent Cells

1) Adherent cells may be cultured on glass chamber slides. Wash in PBS.

2) Fix slides by 2% neutralized formalin for 15 minutes. Rinse slides with PBS then transfer to a Coplin jar containing ice-cold 70% ethanol for 1 hour. Slides may be stored overnight in 70% ethanol at 4°C.

3) Wash in PBS. Inactivate endogenous peroxidases by covering sections with 2% hydrogen peroxide for 5 minutes at room temperature. Wash slides 3×5 minutes with ddH₂O.

4) Follow procedure for pretreatment as required with TdT reaction buffer.

D. Suspension cells

1) Harvest cells and wash them twice in PBS using centrifugation (400g for 5 minutes) to remove residual protein.

2) Adjust the cell concentration to 4–5×10⁶ cells per mL in PBS.

3) Attach cells to slides using either the adhesion or centrifugation method.

a) Adhesion method

1. Clean and label the slides.

2. Wash slides in PBS for 5 minutes at RT.

3. Place slides in a humidified box to prevent them from drying.
4. Place 20-50 μL of the cell suspension (at least enough to cover the well) in each well of the adhesion slides and let cells adhere at room temperature (RT) for 20 minutes.
5. Fix slides by 2% neutralized formalin for 15 minutes. Rinse slides with PBS then transfer to a Coplin jar containing ice-cold 70% ethanol for 1 hour. Slides may be stored overnight in 70% ethanol at 4 °C.
6. Inactivate endogenous peroxidases by covering sections with 2% hydrogen peroxide for 5 minutes at room temperature. Wash slides 3 \times 5 minutes with ddH₂O.
7. Wash in PBS.
8. Follow procedure for pretreatment as required with TdT reaction buffer.

b) Centrifugation method

1. Assemble the CytoSpin centrifuge's sample chamber, filter card, slide, and racks according to the manufacturer's instructions.
2. Load 100 μL of cells in each sample chamber.
3. Centrifuge the slides at 600 rpm for 2-4 minutes.
4. Remove the slides from the rack and place them on a staining rack.
5. Fix slides by 2% neutralized formalin for 15 minutes. Rinse slides with PBS then transfer to a Coplin jar containing ice-cold 70% ethanol for 1 hour. Slides may be stored overnight in 70% ethanol at 4°C.
6. Wash in PBS. Inactivate endogenous peroxidases by covering sections with 2% hydrogen peroxide for 5 minutes at room temperature. Wash slides 3 \times 5 minutes with ddH₂O.
7. Follow procedure for pretreatment as required with TdT reaction buffer.

2. TUNEL Reaction

- 1) Carefully blot away excess water then cover sections with TdT equilibration buffer for 10 minutes at room temperature.
- 2) Preparation of TdT reaction buffer:

	2 samples	4 samples	10 samples
TdT equilibration buffer	24 μL	48 μL	94 μL
TdT enzyme	0.5 μL	1 μL	2.5 μL
Biotinylated dUTP	0.8 μL	1.6 μL	4 μL

Mix well. Prepare fresh from stock solution prior to use.

- 3) Remove TdT equilibration buffer and cover sections with 10 μL of TdT reaction buffer. Incubate slides in a humidified chamber for 30 minutes at 37 °C. In order to conserve reagents a reduced volume of TdT buffer may be carefully covered with a glass coverslip during the incubation. Take care to avoid trapping air bubbles which may lead to staining artifacts.
- 4) Stop reaction by incubating slides 2 \times 10 minutes in 1 \times SSC.

3. Visualized with DAB

- 1) Rinse slides in PBS then block nonspecific binding by covering tissue sections with 2% BSA solution for 10 minutes at room temperature.
- 2) Rinse in PBS for 3 \times 2 minutes.

3) **Detection:** incubate sections with 3-4 drops of RTU streptavidin-HRP for 30 minutes at room temperature.

4) Rinse in PBS for 3×2 minutes.

5) **Chromogen/Substrate:** incubate sections with 3 drops of DAB solution for 2-8 minutes. Monitor signal development under a microscope.

Note: DAB solution is made by mixture of 20 µL of DAB stock solution and 20 µL of stable H₂O₂ solution with 1mL of DAB enhancer buffer (dark-brown stain).

6) Rinse in distilled water 2×2 minutes.

7) **Counterstain:** For using Hematoxylin Nuclear Counterstaining Kit (CAT#: VB-6004), incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tap water 2×2 minutes.

8) Dehydrate through 75% ethanol for 2 minutes, 95% ethanol for 2 minutes, and 100% ethanol for 2×3 minutes. Clear in xylene for 2×5 minutes.

9) Coverslip with mounting medium.

Positive and Negative Controls: As a positive control, treat cells and tissues with DNase I (1µg/mL in 30 mM Tris-HCl (pH 7.2), 140 mM potassium cacodylate, 4 mM MgCl₂, 0.1 mM DTT) for 10 minutes at room temperature. Following DNase I treatment, wash samples 3×2 minutes in DDW then proceed with TUNEL staining. As a negative control, omit the TdT enzyme from the TdT reaction buffer.

Note: This product is intended for research purposes only. This product is **not** intended to be used for therapeutic or diagnostic purposes in humans or animals. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.