DeadEnd™ Fluorometric TUNEL System

INSTRUCTIONS FOR USE OF PRODUCT G3250.
FOR LABORATORY USE.

Quick PROTOCOL

Apoptosis Detection in Adherent Cells

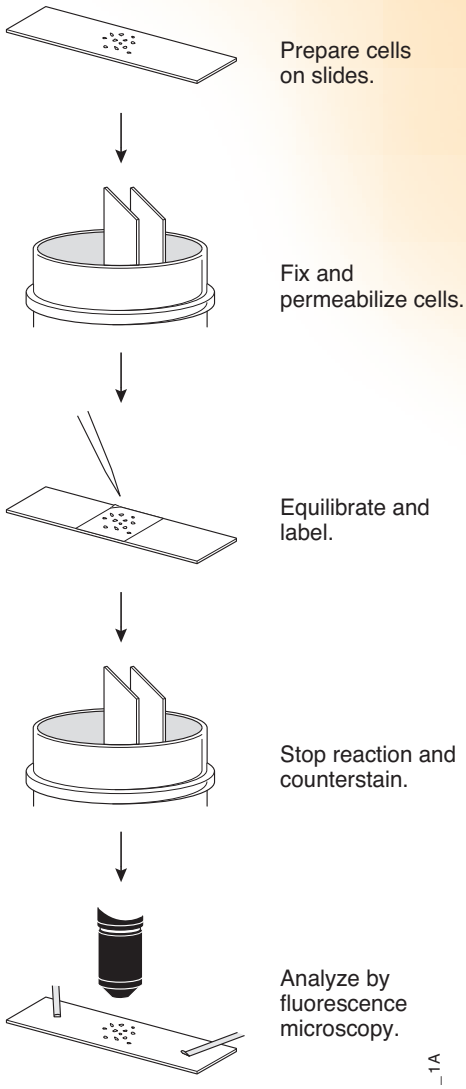
Preparation of Slides

Grow cells on slides or cytospin/pipette cells onto slides.

Apoptosis Detection

1. **Fix:** Immerse slides in 4% formaldehyde in PBS for 25 minutes at 4°C.
2. **Wash:** Immerse slides twice in PBS, 5 minutes each time.
3. **Permeabilize:** Immerse slides in 0.2% Triton® X-100 in PBS for 5 minutes.
4. **Wash:** Immerse slides twice in PBS, 5 minutes each time.
5. **Equilibrate:** Add 100µl Equilibration Buffer. Equilibrate at room temperature for 5–10 minutes.
6. **Label:** Add 50µl of TdT reaction mix to the cells on an area no larger than 5 square centimeters. Do not allow cells to dry completely. Cover slides with Plastic Coverslips to ensure even distribution of the mix. Incubate slides for 60 minutes at 37°C in a humidified chamber; avoid exposure to light from this step forward.
7. **Stop Reaction:** Remove Plastic Coverslips. Immerse slides in 2X SSC for 15 minutes.
8. **Wash:** Immerse slides three times in PBS, 5 minutes each time.
9. **Mount:** Add mounting medium to slides.
10. **Counterstain:** To visualize all nuclei, use Vectashield® with DAPI.
11. **Analyze:** Detect localized green fluorescence of apoptotic cells by fluorescence microscopy. DAPI-stained nuclei will be blue.

For additional protocol information see Technical Bulletin #TB235, available at: **www.promega.com/tbs**



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Apoptosis Detection in Tissue

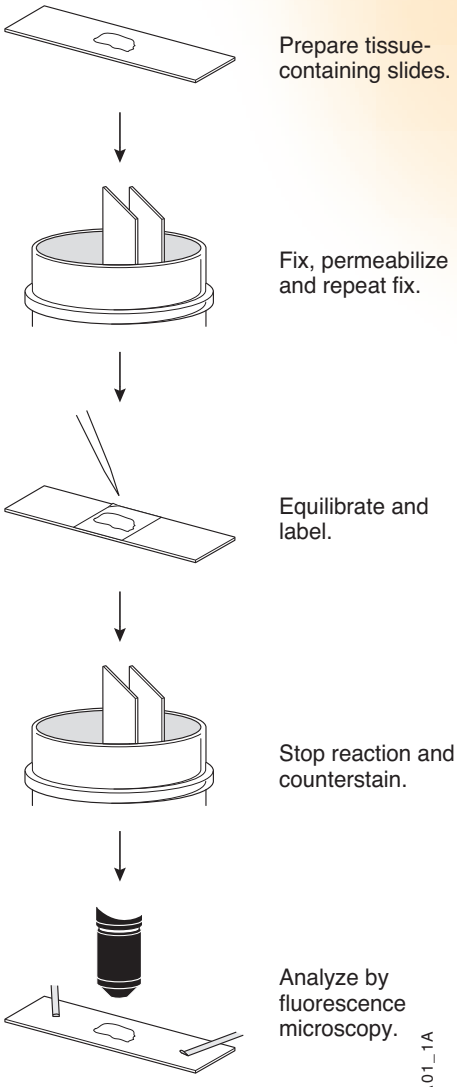
Pretreatment of Paraffin-Embedded Tissue

The following is a standard protocol for tissue sections. Optimization may be required for individual samples.

1. **Remove Paraffin:** Wash slides twice in xylene, 5 minutes each wash.
2. **Wash:** Immerse in 100% ethanol for 5 minutes.
3. **Rehydrate:** Wash slides in decreasing concentrations of ethanol (100%, 95%, 85%, 70%, 50%), 3 minutes each wash.
4. **Wash:** Immerse in 0.85% NaCl for 5 minutes.
5. **Wash:** Immerse in PBS for 5 minutes.

Apoptosis Detection

1. **Fix:** Immerse slides in 4% formaldehyde in PBS for 15 minutes.
2. **Wash:** Immerse slides twice in PBS, 5 minutes each time.
3. **Permeabilize:** Add 100µl of a 20µg/ml Proteinase K solution. Incubate at room temperature for 8–10 minutes. Optimization may be required. **Note:** Longer incubations may be needed for tissue sections thicker than 4–6µm. However, with prolonged Proteinase K incubations, the risk of releasing the tissue sections from the slides increases in subsequent wash steps.
4. **Wash:** Immerse slides in PBS for 5 minutes.
5. **Repeat Fix:** Immerse slides in 4% formaldehyde in PBS for 5 minutes.
6. **Wash:** Immerse slides in PBS for 5 minutes.
7. **Equilibrate:** Add 100µl Equilibration Buffer. Equilibrate at room temperature for 5–10 minutes.
8. **Label:** Add 50µl of TdT reaction mix to the tissue on an area. no larger than 5 square centimeters. Do not allow tissue to dry completely. Cover slides with Plastic Coverslips to ensure even distribution of the mix. Incubate slides for 60 minutes at 37°C in a humidified chamber; avoid exposure to light from this step forward.
9. **Stop Reaction:** Remove Plastic Coverslips. Immerse slides in 2X SSC for 15 minutes.
10. **Wash:** Immerse slides three times in PBS, 5 minutes each time.
11. **Mount:** Add mounting medium to slides.
12. **Counterstain:** To visualize all nuclei, use Vectashield® with DAPI.
13. **Analyze:** Detect localized green fluorescence of apoptotic tissue by confocal fluorescence microscopy. DAPI-stained nuclei will be blue.



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