DeadEnd™ Colorimetric TUNEL System

Instructions for Use of Products G7130 and G7360.
For Laboratory Use.

Quick Protocol

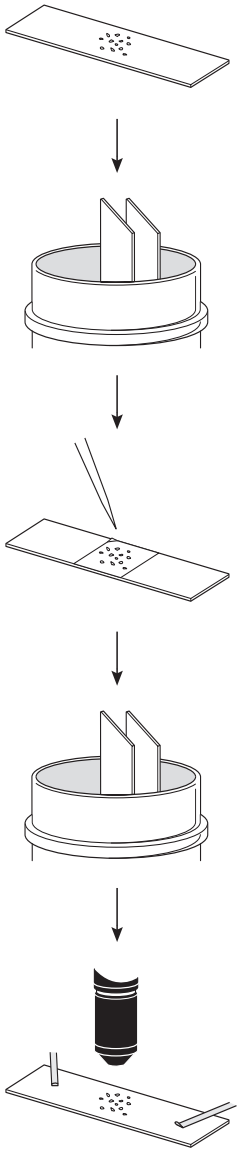
Apoptosis Detection in Cultured Cells

Preparation of Slides

Grow cells on chamber slides or cytospin/pipette cells onto poly-L-lysine-coated slides.

Apoptosis Detection

1. **Fix:** Immerse slides in 10% buffered formalin or 4% paraformaldehyde for 25 minutes.
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 100µl of TdT reaction mix to the cells on the slides. 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.
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. **Block:** Immerse slides in 0.3% hydrogen peroxide for 3–5 minutes.
10. **Wash:** Immerse slides three times in PBS, 5 minutes each time.
11. **Bind:** Add 100µl Streptavidin HRP (diluted 1:500 in PBS). Incubate slides for 30 minutes at room temperature.
12. **Wash:** Immerse slides three times in PBS, 5 minutes each time.
13. **Stain:** Add 100µl DAB Solution (prepare immediately prior to use by adding 100µl DAB 10X Chromogen to 900µl DAB Substrate 1X Buffer). Develop until a light brown background appears. Do not allow the background to become too dark.
14. **Wash:** Immerse slides several times in deionized water.
15. **Visualize:** Mount slides in an aqueous or permanent mounting medium. Observe staining with a light microscope.



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Promega

Quick Protocol

Apoptosis Detection in Tissue Sections

Pretreatment of Tissue Sections

Perform Steps 1–4 for paraffin-embedded tissue sections. For frozen and vibratome tissue sections, perform only Step 4.

1. **Remove Paraffin:** Wash slides twice in xylene, 5 minutes each time.
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 time.
4. **Wash:** Immerse in 0.85% NaCl for 5 minutes. Immerse in PBS for 5 minutes.

Apoptosis Detection

1. **Fix:** Immerse slides in 4% paraformaldehyde 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 10–30 minutes.
4. **Wash:** Immerse slides in PBS for 5 minutes.
5. **Repeat Fix:** Immerse slides in 4% paraformaldehyde in PBS for 5 minutes.
6. **Wash:** Immerse slides twice in PBS, 5 minutes each time.
7. **Equilibrate:** Add 100µl Equilibration Buffer. Equilibrate at room temperature for 5–10 minutes.
8. **Label:** Add 100µl of TdT reaction mix to the tissue sections on the slides. Do not allow tissue sections 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.
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. **Block:** Immerse slides in 0.3% hydrogen peroxide for 3–5 minutes.
12. **Wash:** Immerse slides three times in PBS, 5 minutes each time.
13. **Bind:** Add100µl Streptavidin HRP (diluted 1:500 in PBS). Incubate slides for 30 minutes at room temperature.
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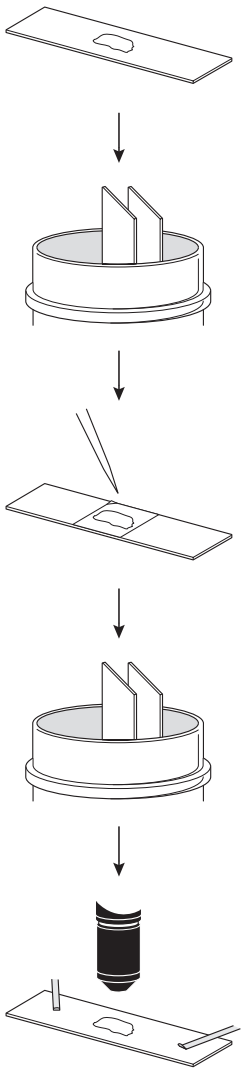
15. **Stain:** Add 100µl DAB Solution (prepare immediately prior to use by adding 100µl DAB 10X Chromogen to 900µl DAB Substrate 1X Buffer). Develop until a light brown background appears. Do not allow the background to become too dark.
16. **Wash:** Immerse slides several times in deionized water.
17. **Visualize:** Mount slides in an aqueous or permanent mounting medium. Observe staining with a light microscope.



Additional protocol information in Technical Bulletin #TB199, available online at: **www.promega.com**

PROMEGA CORPORATION • 2800 WOODS HOLLOW ROAD • MADISON, WI 53711-5399 USA • TELEPHONE 608-274-4330
www.promega.com