

1.2.2 TUNEL protocol for tissues which tend to give false positives

The protocol given below has been found to eliminate the TUNEL labeling “false positives” seen with certain paraffin-embedded tissue sections (for example, of rabbit endometrium). The key step is pretreatment of the slide with microwave irradiation rather than proteinase K.

Sample: Paraffin-embedded tissue sections (e.g., of rabbit endometrium)

Reagents: *In Situ* Cell Death Detection Kit, POD, Cat. No. 11 684 817 001
DAB Substrate, Cat. No. 11 718 096 001

- 1 Dewax paraformaldehyde- or formalin-fixed tissue sections according to standard procedures.
- 2 Place the slide(s) in a plastic jar containing 200 ml 0.1 M citrate buffer, pH 6.0, put the jar in a microwave oven, and apply 750 W (high) microwave irradiation for 1 min. For rapid cooling, immediately add 80 ml redist. water (20°–25°C) to the jar, then transfer the slide(s) into PBS (20°–25°C).
Caution: DO NOT perform a proteinase K treatment!
- 3 Immerse the slide(s) for 30 min at room temperature (RT) in a blocking solution containing 0.1 M Tris-HCl, 3% BSA, and 20% normal bovine serum, pH 7.5.
- 4 Rinse the slide(s) twice with PBS at RT. Let excess fluid drain off.
- 5 Apply 50 µl of TUNEL reaction mixture to the section and incubate for 60 min at 37°C in a humidified atmosphere.
- 6 Rinse slide(s) three times in PBS (5 min for each wash).
Note: At this stage, you can evaluate the section under a fluorescence microscope.
- 7 Block endogenous peroxidase activity by incubating slides for 10 min at RT with 0.3% H₂O₂ in methanol.
- 8 Repeat steps 3 and 4 to block nonspecific binding of the anti-fluorescein-antibody to the tissue.
- 9 Add 50 µl Converter-POD, pre-diluted 1:2 in blocking solution (from Step 3), and incubate for 30 min at 37°C in a humidified atmosphere.
- 10 Rinse slide(s) three times in PBS at RT for 5 min each.
- 11 Add 50 µl DAB substrate solution and incubate for 1–3 min at RT.
- 12 Wash slide(s) extensively in tap water and counterstain if needed.

