

In Situ Cell Death Detection Kit, AP

Cat. No. 11 684 809 001 50 tests

In Situ Cell Death Detection Kit, POD

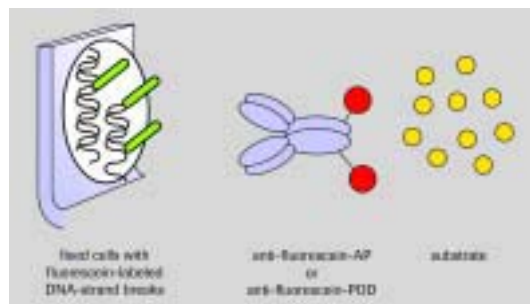
Cat. No. 11 684 817 001 50 tests

Type	Indirect TUNEL labeling assay
Useful for	Detection of DNA strand breaks in apoptotic cells under a light microscope
Samples	Cell smears, adherent cells, cytopins, frozen or fixed tissue sections
Method	End-labeling of DNA with fluorescein-dUTP, followed by detection of incorporated fluorescein with an antibody and visualization of the antibody
Time	Approx. 3 h (+ sample preparation, permeabilization, etc.)

Significance of kits: These two *In Situ* Cell Death Detection Kits measure cell death (apoptosis) by detecting DNA strand breaks in individual cells by light microscopy. The AP and POD kits offer an indirect TUNEL detection method, which is a fast, sensitive, and specific light microscopic assay.

Test principle: The assays use an optimized terminal transferase (TdT) to label free 3'OH ends in genomic DNA with fluorescein-dUTP. The procedure involves:

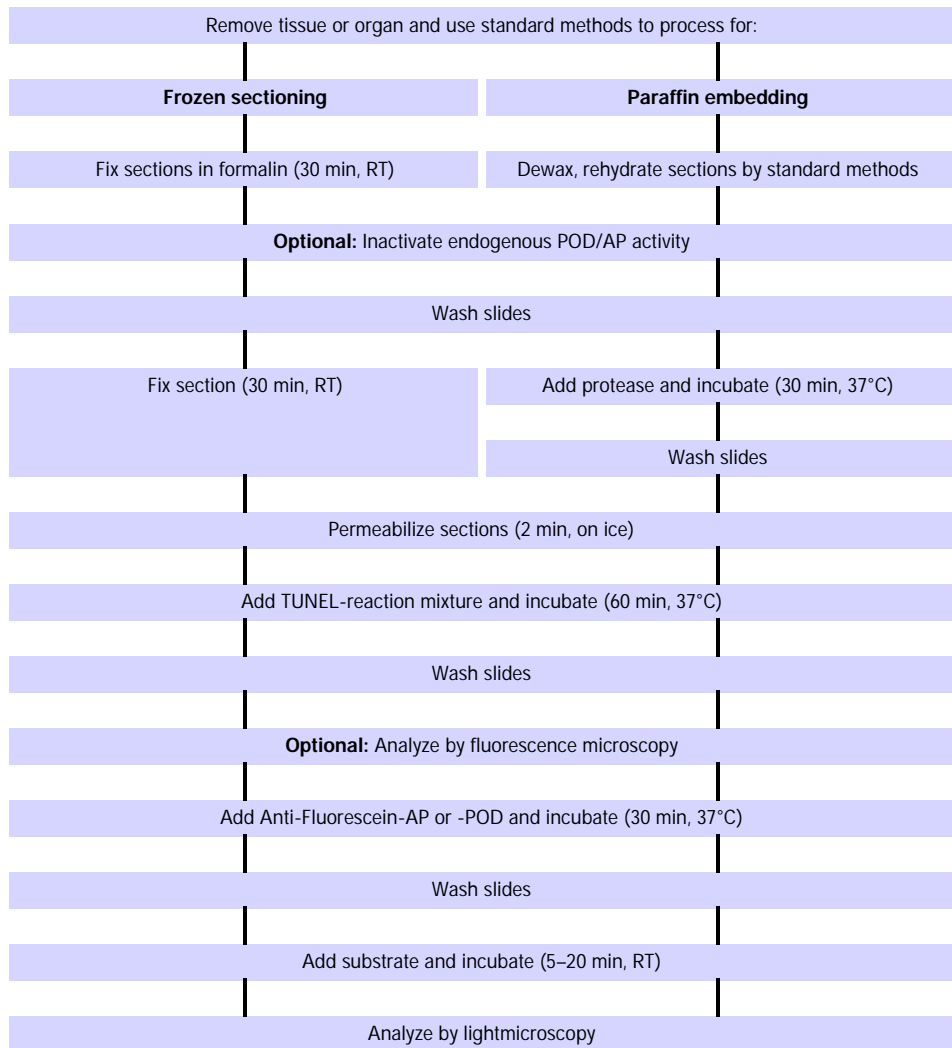
- 1 Fixing and permeabilizing apoptotic cells or tissue sections.
- 2 Incubating the cells with the TUNEL reaction mixture containing TdT and fluorescein-dUTP. During this incubation step, TdT catalyzes the attachment of fluorescein-dUTP to free 3'OH ends in the DNA.
- 3 Detecting the incorporated fluorescein with an anti-fluorescein antibody AP conjugate (*In Situ* Cell Death Detection Kit, AP) or an anti-fluorescein antibody POD conjugate (*In Situ* Cell Death Detection Kit, POD).
- 4 Visualizing the immunocomplexed AP or POD with a substrate reaction.



◀ Figure 29: Schematic showing the principle of the *In Situ* Cell Death Detection Kits, AP and POD.

Sensitivity: The enzymatic labeling allows the detection of an apoptotic event that occurs, prior to changes in morphology and even before DNA fragments become detectable in the cytoplasm²². It detects early stage of DNA fragmentation in apoptotic cells. This is especially important if apoptosis is studied *in vivo*, e.g., in tissue sections, since apoptotic cells are rapidly and efficiently removed *in vivo*.

Specificity: The amount of DNA strand breaks in apoptotic cells is so large that the degree of cell labeling in these assays is an adequate discriminator between apoptotic and necrotic cells¹⁹.



▲ Flow Chart 8: Assay procedure *In Situ* Cell Death Detection Kits (AP or POD).

Can be used to assay:

- Cells smears, adherent cells
- Cytospins
- Tissue sections (frozen or paraffin-embedded).

Kit contents

***In Situ* Cell Death Detection Kit, AP**

1. Enzyme solution (TdT), 5 tubes
2. Labeling solution (nucleotide mix), 5 tubes
3. Anti-Fluorescein-AP conjugate, ready to use

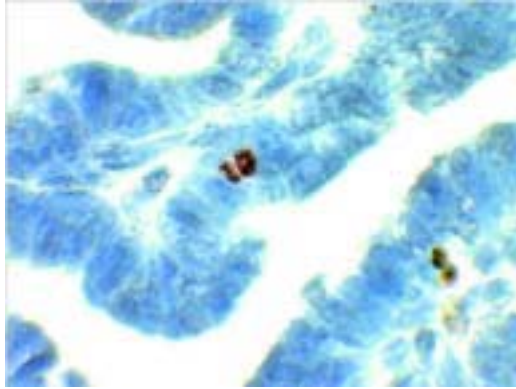
***In Situ* Cell Death Detection Kit, POD**

1. Enzyme solution (TdT), 5 tubes
2. Labeling solution (nucleotide mix), 5 tubes
3. Anti-Fluorescein-POD conjugate, ready to use

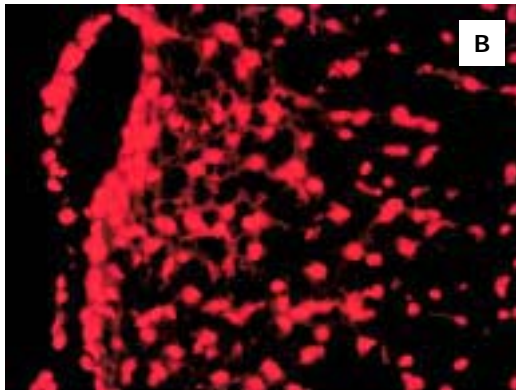
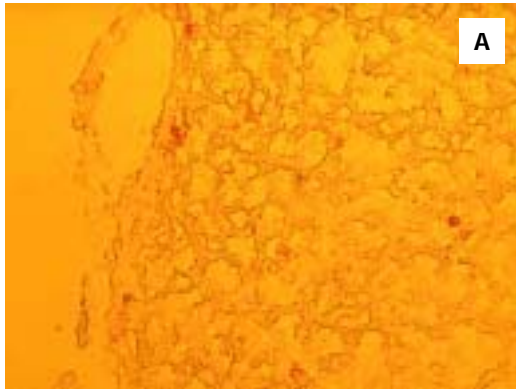
Note: For added flexibility and convenience, the components of these kits, as well as several AP and POD precipitating substrates are also available as single reagents (Table 6).

Typical results: see Figures 30 and 31.

Technical tips: For more information on the use of the kits for light microscopic analysis, see pages 113–116 in the Appendix, of this guide.



▲ **Figure 30: Detection of apoptotic cells by TUNEL and peroxidase staining in rabbit endometrium.** A tissue section from rabbit endometrium was prepared and assayed with the *In Situ* Cell Death Detection Kit, POD. Slide was counterstained with hematoxylin and viewed under a light microscope.
Result: A subpopulation of apoptotic cells, scattered throughout the tissue section, are intensely stained (brown) by the TUNEL treatment and subsequent peroxidase immunostaining.



▲ **Figure 31: Detection of apoptotic cells by TUNEL and alkaline phosphatase staining in rat spinal cord.** A tissue section from rat spinal cord was prepared and assayed with the *In Situ* Cell Death Detection Kit, AP. The slide was viewed under a light microscope (Panel A). After viewing, the same slide was stained with propidium iodide and viewed by fluorescence microscopy (Panel B).
Result: A few apoptotic cells (red) are clearly visible after TUNEL treatment and subsequent alkaline phosphatase immunostaining (Panel B). However, the apoptotic cells are not visible in the same slide after staining with propidium iodide (Panel A).

Other applications: For more examples of how the *In Situ* Cell Death Detection Kits (AP or POD) can be used in the lab, see Appendix, pages 136–138.

For your convenience, we offer a number of additional single reagents to optimize your TUNEL reaction (Table 6)

Product	Cat. No.	Pack Size
TUNEL Label Mix	11 767 291 001	3 x 550 µl (30 tests)
TUNEL Enzyme	11 767 305 001	2 x 50 µl (20 tests)
TUNEL POD (Anti-Fluorescein, POD conjugate)	11 772 465 001	3.5 ml (70 tests)
TUNEL AP (Anti-Fluorescein, AP conjugate)	11 772 457 001	3.5 ml (70 tests)
TUNEL Dilution Buffer	11 966 006 001	2 x 10 ml
DAB Substrate, metal enhanced, precipitating (Peroxidase (POD) substrate)	11 718 096 001	1 pack
NBT/BCIP Stock Solution (AP substrate)	11 681 451 001	8 ml
Fast Red Tablets (AP substrate)	11 496 549 001	20 tablets

▲ Table 6: Single reagents available for the TUNEL technique.

