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In Situ Cell Death Detection Kit, Fluorescein

Cat. No. 11 684 795 001 50 tests

In Situ Cell Death Detection Kit, TMR red

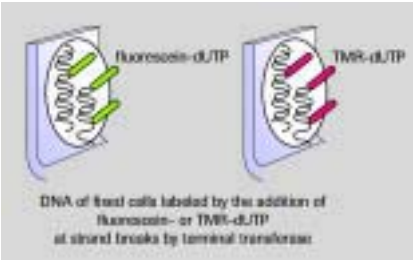
Cat. No. 12 156 792 001 50 tests

Type	Direct TUNEL labeling assay
Useful for	Detection of DNA strand breaks in apoptotic cells by flow cytometry or fluorescence microscopy
Samples	Cells in suspension, adherent cells, cell smears, frozen or paraffin-embedded tissue sections
Method	End-labeling of DNA with fluorescein-dUTP or tetramethylrhodamine-dUTP (TMR-dUTP), followed by direct analysis of fluorescent cells
Time	1–2 h (+ sample preparation, permeabilization, etc.)

Significance of kit: This two *In Situ* Cell Death Detection Kits, measure and quantitate cell death (apoptosis) by labeling and detection of DNA strand breaks in individual cells by flow cytometry or fluorescence microscopy. The kits offer a direct TUNEL detection method, for maximum sensitivity and minimal background.

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

1. Fixing and permeabilizing apoptotic cells.
2. Incubating the cells with the TUNEL reaction mixture containing TdT and fluorescein-dUTP or TMR-dUTP. During this incubation step, TdT catalyzes the attachment of fluorescein-dUTP or TMR-dUTP to free 3'OH ends in the DNA.
3. Visualizing the incorporated fluorescein with a flow cytometer and/or a fluorescence microscope (fluorescein/TMR red).

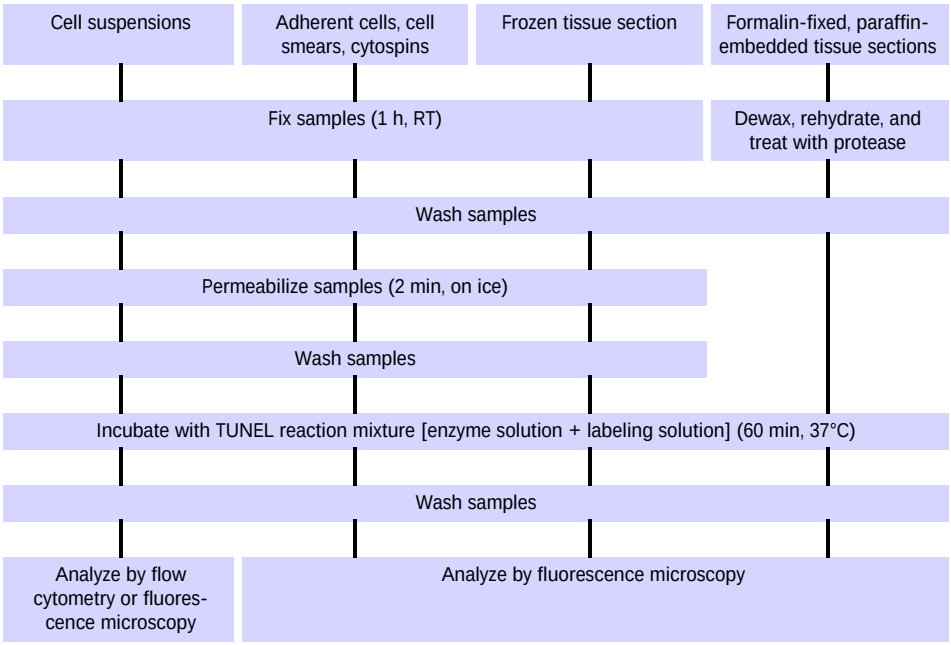


▲ **Figure 24: Schematic showing the principle of the *In Situ* Cell Death Detection Kits, Fluorescein and TMR red.**

For a detailed overview of the steps in the procedure, see Flow Chart 7.

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 cytoplasm[22]. 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 cells[19].



▲ **Flow Chart 7: Assay procedure, *In Situ* Cell Death Detection Kits (Fluorescein or TMR red).**

Can be used to assay:

- Cells in suspension (permanent cell lines, normal and tumor cells *ex vivo*)
- Cytospins, cell smears
- Adherent cells cultured on chamber slides
- Frozen tissue sections
- Formalin-fixed, paraffin-embedded tissue sections

Kit contents

1. Enzyme solution (TdT), 5 tubes
2. Labeling solution (nucleotide mix), 5 tubes

Typical results: See Figures 25–28.

Technical tips: For more information on the use of the kit for flow cytometric analysis, see page 119 in the Appendix of this guide.

Other applications: For more examples of how the *In Situ* Cell Death Detection Kits (Fluorescein or TMR red) can be used in the lab, see Appendix, pages 134–136.

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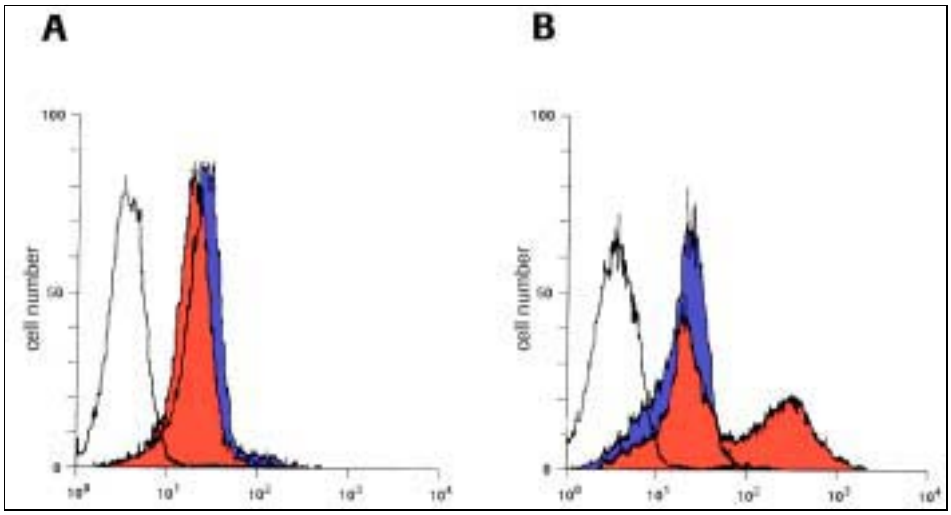
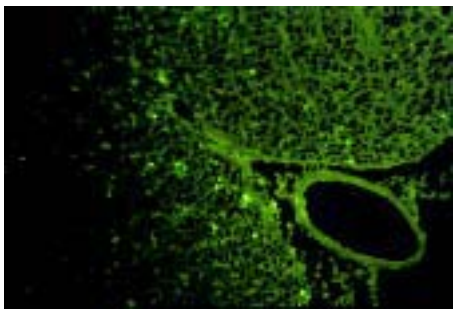
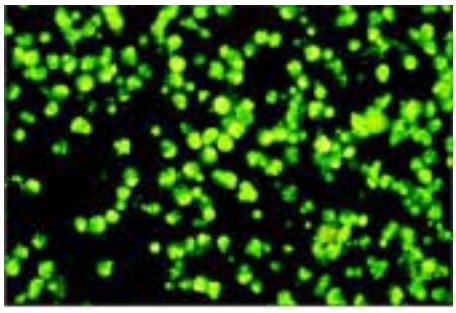


▲ **Figure 25: Detection of apoptotic cells by flow cytometry using the *In Situ* Cell Death Detection Kit, Fluorescein.** HL60 cells were cultured in the absence (A) or presence (B) of 2 µg/ml Camptothecin for 3 h at 37°C. Cells were incubated either with TUNEL reaction mixture (■) or label solution as negative control (■) or PBS for autofluorescence (□).



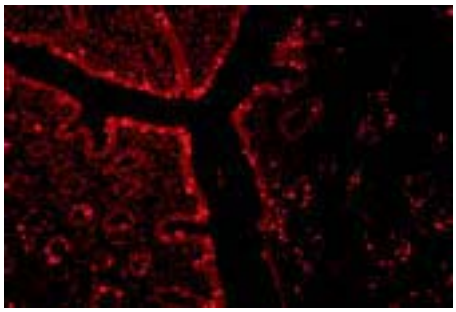
◀ **Figure 26: Detection of apoptotic cells (green) by fluorescence microscopy in a tissue section from rat.** A tissue section from a rat spinal cord was prepared and assayed with the *In Situ* Cell Death Detection Kit, Fluorescein. The treated section was viewed under a fluorescence microscope. (Photomicrograph was kindly provided by R. Gold, University of Würzburg, Germany.)

Result: A subpopulation of apoptotic cells, scattered throughout the tissue section, are intensely stained (green) by the TUNEL treatment and are easily visible under the microscope.



◀ **Figure 27: Cell suspension stained with the *In Situ* Cell Death Detection Kit, Fluorescein.** U937 cells induced with 4 µg/ml camptothecin, showing positive staining of apoptotic nuclei.

Note: This figure shows a high number (>80%) of apoptotic cells. To avoid detecting cells that are undergoing secondary necrosis, analyze cells earlier in the process after induction of apoptosis.



◀ **Figure 28: Rabbit endometrium, stained with the *In Situ* Cell Death Detection Kit, TMR red, and viewed under a fluorescence microscope.** Apoptotic nuclei stain bright red, limited fluorescence is visible in background tissue.