For life science research only.
Not for use in diagnostic procedures.

Print Protocol

Print Changes to Previous Version

In Situ Cell Death Detection Kit, POD

Version 15

Content version: May 2016

Kit for immunohistochemical detection and quantification of apoptosis (programmed cell death) at single cell level, based on labeling of DNA strand breaks (TUNEL technology): Analysis by light microscopy.

Cat. No. 11 684 817 910 1 Kit (50 tests)

Store the kit at −15 to −25°C

1. Preface

1.1 Table of contents

Caution

The Label solution contains cacodylate, toxic by inhalation and swallowed, and cobalt dichloride, which may cause cancer by inhalation. Avoid exposure and obtain special instructions before use.

When using do not eat, drink or smoke. After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell seek medical advice immediately (show label where possible).
Collect the supernatants from the labeling reactions in a tightly closed, non-breakable container and indicate contents. Discard as regulated for toxic waste.

Note: In contrast to preceding kits/vials, now the Enzyme Solution does no longer contain potassium cacodylate. Thus vial 1 is not toxic.

Kit contents

Please refer to the following table for the contents of the kit.

Vial/ Cap	Label	Contents
1 blue	Enzyme Solution	• Terminal deoxynucleotidyl transferase from calf thymus *(EC 2.7.7.31)*, recombinant in *E. coli,* in storage buffer • 10× conc. • 5 × 50 µl
2 violet	Label Solution	• Nucleotide mixture in reaction buffer • 1× conc. • 5 × 550 µl
3 yellow	Converter-POD	• Anti-fluorescein antibody, Fab fragment from sheep, conjugated with horse-radish peroxidase (POD) • Ready-to-use • 3.5 ml

Additional equipment required

In addition to the reagents listed above, you have to prepare several solutions. In the table you will find an overview about the equipment which is needed for the different procedures.

Detailed information is given in front of each procedure.

Procedure	Equipment	Reagents
Preparation of sample material (section 3.2)		
• Adherent cells, cell smears and cytospin preparations (section 3.2.1) • Cryopreserved tissue (section 3.2.2.2)		• Washing buffer: Phosphate buffered saline (PBS*) • Blocking solution: 3% H_2O_2 in methanol • Fixation solution: 4% Paraformaldehyde in PBS, pH 7.4, freshly prepared • Permeabilisation solution: 0.1% Triton X-100 in 0.1% sodium citrate, freshly prepared (6)

Paraffin-embedded tissue (section 3.2.2.1)		• Xylene and ethanol (absolute, 95%, 90%, 80%, 70%, diluted in double distilled water) • Washing buffer: PBS* • Proteinase K*, PCR Grade, working solution: [10 - 20 µg/ml in 10 mM Tris/HCl, pH 7.4-8] Alternative treatments • Permeabilisation solution: (0.1% Triton X–100, 0.1% sodium citrate) , freshly prepared • Pepsin* (0.25% - 0.5% in HCl, pH 2) or trypsin*, 0.01 N HCl • 0.1 M Citrate buffer, pH 6 for microwave irradiation
Labeling protocol (section 3.3)		
Positive control (section 3.3.1)		• Micrococcal nuclease or • DNase I, recombinant, grade I *
Adherent cells, cell smears, cytospin preparations, and tissues (section 3.3.2)	• Parafilm or coverslips • Humidified chamber	Washing buffer: PBS*
Difficult tissue (section 3.3.3)	• Plastic jar • Microwave • Humidified chamber	• Citrate buffer, 0.1 M, pH 6.0. • Washing buffer: PBS* • Tris-HCl, 0.1 M pH 7.5, containing 3% BSA* and 20% normal bovine serum
Signal conversion (section 3.4)		
	• Humidified chamber • Parafilm or coverslip	• Washing buffer: PBS* • DAB Metal Enhanced Substrate Set* or alternative POD substrates • Mounting medium for light microscopy

2. Introduction

2.1 Product overview

Test principle

Cleavage of genomic DNA during apoptosis may yield double-stranded, low molecular weight DNA fragments (mono- and oligonucleosomes) as well as single strand breaks ("nicks") in high molecular weight DNA.

Those DNA strand breaks can be identified by labeling free 3'-OH termini with modified nucleotides in an enzymatic reaction.

Stage	Description
1	Labeling of DNA strand breaks, by Terminal deoxynucleotidyl transferase (TdT), which catalyzes polymerization of labeled nucleotides to free 3'-OH DNA ends in a template-independent manner (TUNEL-reaction).
2	Incorporated fluorescein is detected by anti-fluorescein antibody Fab fragments from sheep, conjugated with horse-radish peroxidase (POD). After substrate reaction, stained cells can be analyzed under light microscope.
3	After substrate reaction, stained cells can be analyzed under light microscope.

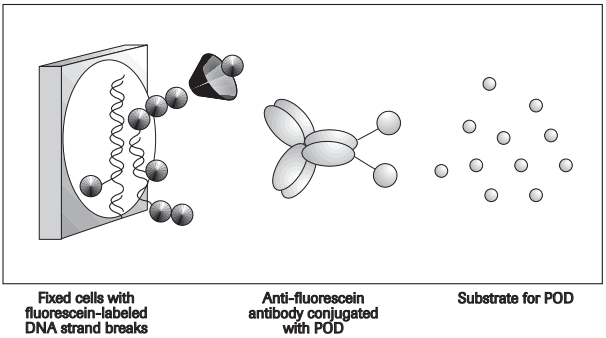


Fig. 1: Test principle

Application

The *In Situ* Cell Death Detection Kit is designed as a precise, fast and simple, non-radioactive technique to detect and quantify apoptotic cell death at single cell level in cells and tissues. Thus, the *In Situ* Cell Death Detection Kit can be used in many different assay systems. Examples are:

- Detection of individual apoptotic cells in frozen and formalin fixed tissue sections in basic research and routine pathology.
- Determination of sensitivity of malignant cells to drug induced apoptosis in cancer research.
- Typing of cells undergoing cell death in heterogeneous populations by double staining procedures (6).

Specificity

The TUNEL reaction preferentially labels DNA strand breaks generated during apoptosis. This allows discrimination of apoptosis from necrosis and from primary DNA strand breaks induced by cytostatic drugs or irradiation (3, 4).

Test interference

False negative results: DNA cleavage can be absent or incomplete in some forms of apoptotic cell death (37). Sterical hindrance such as extracellular matrix components can prevent access of TdT to DNA strand breaks. In either case false negative results can be obtained.

False positive results: Extensive DNA fragmentation may occur in late stages of necrosis (4, 38).

DNA strand breaks may also be prominent in cell populations with high proliferative or metabolic activity. In either case false positive results may be obtained. To confirm apoptotic mode of cell death, the morphology of respective cells should be examined very carefully. Morphological changes during apoptosis have a characteristic pattern. Therefore evaluation of cell morphology is an important parameter in situations where there is any ambiguity regarding interpretation of results.

Sample material

- Cytospin and cell smear preparations
- Adherent cells cultured on chamber slides (31)
- Frozen or formalin-fixed, paraffin-embedded tissue sections (1, 25, 26, 29, 30, 32–34, 36, 39)

Assay time

2–3 hours, excluding culture, fixation and permeabilisation of cells and preparation of tissue sections.

Number of tests

The kit is designed for 50 tests.

Kit storage/stability

The unopened kit is stable at −15 to −25°C until the expiration date printed on the label.

Reagent	Storage and stability
TUNEL reaction mixture	The TUNEL reaction mixture should be prepared immediately before use and should not be stored. Keep TUNEL reaction mixture on ice until use.
Converter-POD	Once thawed the Converter-POD solution should be stored at +2 to +8°C (maximum stability 6 months). ***Note***: Do not freeze!

Advantage

Please refer to the following table.

Benefit	Feature
Sensitive	Detection of apoptotic cell death at single cell level at very early stages (1, 2, 6).
Specific	Preferential labeling of apoptosis versus necrosis (3, 4).
Fast	Short assay time (2-3 h).
Convenient	• Reagents are provided in stable, optimized form. • No dilution steps required.
Flexible	• Suitable for fixed cells and tissue. This allows accumulation, storage and transport of samples (2, 5). • Double staining enables identification of type and differentiation state of cells undergoing apoptosis (6).
Function-tested	Every lot is function-tested on apoptotic cells in comparison to a master lot.

Cell death

Two distinct modes of cell death, apoptosis and necrosis, can be distinguished based on differences in morphological, biochemical and molecular changes of dying cells.

Programmed cell death or apoptosis is the most common form of eukaryotic cell death. It is a physiological suicide mechanism that preserves homeostasis, in which cell death naturally occurs during normal tissue turnover (8, 9). In general, cells undergoing apoptosis display a characteristic pattern of structural changes in nucleus and cytoplasm, including rapid blebbing of plasma membrane and nuclear disintegration. The nuclear collapse is associated with extensive damage to chromatin and DNA-cleavage into oligonucleosomal length DNA fragments after activation of a calcium-dependent endogenous endonuclease (10, 11). However, very rare exceptions have been described where morphological features of apoptosis are not accompanied with oligonucleosomal DNA cleavage (37).

Apoptosis

Apoptosis is essential in many physiological processes, including maturation and effector mechanisms of the immune system (12, 13), embryonic development of tissue, organs and limbs (14), development of the nervous system (15, 16) and hormone-dependent tissue remodeling (17). Inappropriate regulation of apoptosis may play an important role in many pathological conditions like ischemia, stroke, heart disease, cancer, AIDS, autoimmunity, hepatotoxicity and degenerative diseases of the central nervous system (18–20).

In oncology, extensive interest in apoptosis comes from the observation, that this mode of cell death is triggered by a variety of antitumor drugs, radiation and hyperthermia, and that the intrinsic propensity of tumor cells to respond by apoptosis is modulated by expression of several oncogenes (21).

Identification of apoptosis

Several methods have been described to identify apoptotic cells (22– 24). Endonucleolysis is considered as the key biochemical event of apoptosis, resulting in cleavage of nuclear DNA into oligonucleosome-sized fragments. Therefore, this process is commonly used for detection of apoptosis by the typical “DNA ladder“ on agarose gels during electrophoresis. This method, however, can not provide information regarding apoptosis in individual cells nor relate cellular apoptosis to histological localization or cell differentiation.

This can be done by enzymatic *in situ* labeling of apoptosis induced DNA strand breaks. DNA polymerase as well as terminal deoxynucleotidyl transferase (TdT) (1-6, 25-36, 41) have been used for the incorporation of labeled nucleotides to DNA strand breaks *in situ*. The tailing reaction using TdT, which was also described as ISEL (*in situ* end labeling) (5, 35) or TUNEL (TdT-mediated dUTP nick end labeling) (1, 6, 31, 33) technique, has several advantages in comparison to the *in situ* nick translation (ISNT) using DNA polymerase:

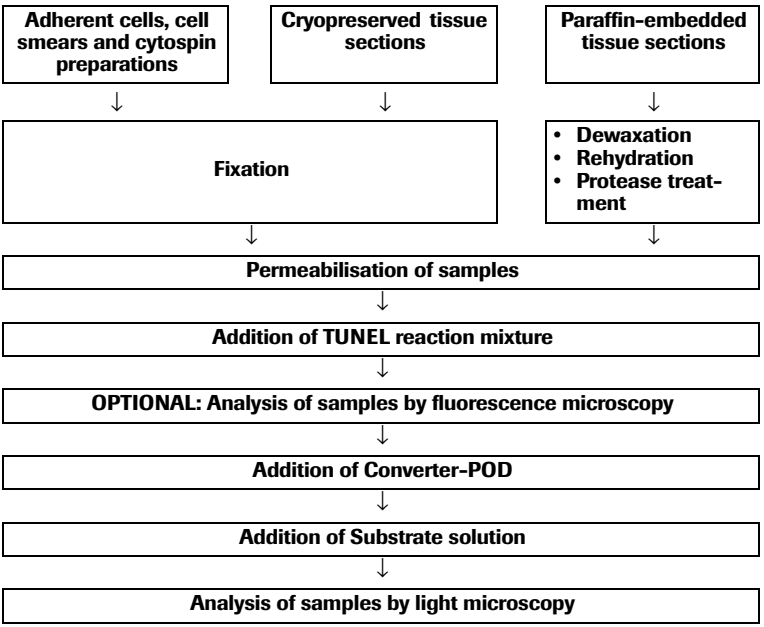
- Label intensity of apoptotic cells is higher with TUNEL compared to ISNT, resulting in an increased sensitivity (2, 4).
- Kinetics of nucleotide incorporation is very rapid with TUNEL compared to the ISNT (2, 4).
- TUNEL preferentially labels apoptosis in comparison to necrosis, thereby discriminating apoptosis from necrosis and from primary DNA strand breaks induced by antitumor drugs or radiation (3, 4).

The working procedure described below has been developed and published by R. Sgonc and colleagues (6). The main advantage of this simple and rapid procedure is the use of fluorescein-dUTP to label DNA strand breaks. This allows the detection of DNA fragmentation by fluorescence microscopy **directly after the TUNEL reaction** prior to the addition of the secondary anti-fluorescein-POD-conjugate.

3.1 Flow chart

Assay procedure

The assay procedure is explained in the following flow chart.



3.2 Preparation of sample material

3.2.1 Adherent cells, cell smears and cytospin preparations

Additional solutions required

- Washing buffer: Phosphate buffered saline (PBS)
- Blocking solution: 3% H_2O_2 in methanol
- Fixation solution: 4% Paraformaldehyde in PBS, pH 7.4, freshly prepared
- Permeabilisation solution: 0.1% Triton X-100 in 0.1% sodium citrate, freshly prepared (6)

Procedure

In the following table describes the fixation of cells, blocking of endogenous peroxidase and cell permeabilisation.

Note: Fix and permeabilisate two additional cell samples for the negative and positive labeling controls.

Step	Action
1	Fix air dried cell samples with a freshly prepared **Fixation solution** for 1 h at +15 to +25°C.
2	Rinse slides with **PBS.**
3	Incubate with **Blocking solution** for 10 min at +15 to +25°C.
4	Rinse slides with **PBS**.
5	Incubate in **Permeabilisation solution** for 2 min on ice (+2 to +8°C).
6	Proceed as described under 3.3.

3.2.2.1 Treatment of paraffin-embedded tissue

Pretreatment of paraffin embedded tissue

Tissue sections can be pretreated in 4 different ways. If you use Proteinase K, PCR Grade, the concentration, incubation time, and temperature have to be optimized for each type of tissue (1, 29, 33, 36, 40, 42).

Note: Use Proteinase K, PCR Grade, only from Roche Applied Science, because it is tested for absence of nucleases, according to the current quality control procedures, which might lead to false-positive results!

The other 3 alternative procedures are also described in the following table (step 2).

Additional solutions required

- Xylene and ethanol (absolute, 95%, 90%, 80%, 70%, diluted in double distilled water)
- Washing buffer: PBS
- Proteinase K, PCR Grade*, working solution: [10-20 µg/ml in 10 mM Tris/HCl, pH 7.4-8]

Alternative treatments

- Permeabilisation solution: 0.1% Triton X–100, 0.1% sodium citrate, freshly prepared
- Pepsin* (0.25% - 0.5% in HCl, pH 2) or trypsin*, 0.01 N HCl
- 0.1 M Citrate buffer, pH 6 for the microwave irradiation

Procedure

In the following table the pretreatment of paraffin-embedded tissue with Proteinase K, PCR Grade treatment and 3 alternative procedures are described.

Note: Add additional tissue sections for the negative and positive labeling controls.

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Dewax and rehydrate tissue section according to standard protocols (e.g., by heating at 60°C followed by washing in xylene and rehydration through a graded series of ethanol and double dist. water) (1, 33, 36).</td></tr>
<tr><td>2</td><td>Incubate tissue section for 15 - 30 min at +21 to +37°C with Proteinase K working solution.
<table>
<tr><th>Alternatives:</th><th>Treatment:</th></tr>
<tr><td>1. Permeabilisation solution</td><td>Incubate slides for 8 min.</td></tr>
<tr><td>2. Pepsin* (30, 40) or trypsin*</td><td>15-60 min at +37°C.</td></tr>
<tr><td>3. Microwave irradiation</td><td>• Place the slide(s) in a plastic jar containing 200 ml 0.1 M Citrate buffer, pH 6.0.
• Apply 350 W microwave irradiation for 5 min.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Proceed as described under 3.3.</td></tr>
</table>

3.2.2.2 Treatment of cryopreserved tissue

Additional solutions required

- Fixation solution: 4% Paraformaldehyde in PBS, pH 7.4, freshly prepared
- Washing buffer: PBS
- Blocking solution: 3% H_2O_2 in methanol
- Permeabilisation solution (0.1% Triton X–100, 0.1% sodium citrate), freshly prepared

Cryopreserved tissue

In the following table the pretreatment of cryopreserved tissue is described.

Note: Fix and permeabilisate two additional samples for the negative and positive labeling controls.

Step	Action
1	Fix tissue section with **Fixation solution** for 20 min at +15 to +25°C.
2	Wash 30 min with **PBS**. ***Note***: For storage, dehydrate fixed tissue sections 2 min in absolute ethanol and store at −15 to −25°C.
3	Incubate with **Blocking solution** for 10 min at +15 to +25°C.
4	Rinse slides with **PBS**.
5	Incubate in **Permeabilisation solution** for 2 min on ice (+2 to +8°C).
6	Proceed as described under 3.3.

3.3.1 Before you begin

Preparation of TUNEL reaction mixture

One pair of tubes (vial 1: Enzyme Solution, and vial 2: Label Solution) is sufficient for staining 10 samples by using 50 µl TUNEL reaction mixture per sample and 2 negative controls by using 50 µl Label Solution per control.

Note: The TUNEL reaction mixture should be prepared immediately before use and should not be stored. Keep TUNEL reaction mixture on ice until use.

Step	Action
1	Remove 100 µl **Label Solution** (vial 2) for two negative controls.
2	Add total volume (50 µl) of **Enzyme solution** (vial 1) to the remaining 450 µl Label Solution in vial 2 to obtain 500 µl TUNEL reaction mixture.
3	Mix well to equilibrate components.

Additional reagents required

- Micrococcal nuclease or
- DNase I recombinant, grade I *

Controls

Two negative controls and a positive control should be included in each experimental set up.

Negative control:	Incubate fixed and permeabilized cells in 50 µl/well **Label Solution** (without terminal transferase) instead of TUNEL reaction mixture.
Positive control:	Incubate fixed and permeabilized cells with **micrococcal nuclease or DNase I recombinant**, grade I (3,000 U/ml– 3 U/ml in 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ 1 mg/ml BSA) for 10 min at +15 to +25°C to induce DNA strand breaks, prior to labeling procedures.

3.3.2 Labeling protocol for adherent cells, cell smears, cytospin preparations and tissues

Additional equipment and solutions required

- Washing buffer: PBS
- Humidified chamber
- Parafilm or coverslip

Procedure

Please refer to the following table.

Step	Action
1	Rinse slides twice with **PBS**.
2	Dry area around sample.
3	Add 50 µl **TUNEL reaction mixture** on sample. ***Note***: For the negative control add 50 µl Label solution each. To ensure a homogeneous spread of TUNEL reaction mixture across cell monolayer and to avoid evaporative loss, samples should be covered with parafilm or coverslip during incubation.
4	Add lid and incubate for 60 min at +37°C in a humidified atmosphere in the dark.
5	Rinse slide 3 times with **PBS**.
6	Samples can be analyzed in a drop of PBS under a fluorescence microscope at this state. Use an excitation wavelength in the range of 450–500 nm and detection in the range of 515 – 565 nm (green).

3.3.3 Labeling protocol for difficult tissue

Additional equipment and solutions required

- Citrate buffer, 0.1 M, pH 6.0.
- Washing buffer: PBS
- Tris-HCl, 0.1 M pH 7.5, containing 3% BSA and 20% normal bovine serum
- Plastic jar
- Microwave
- Humidified chamber

Procedure

Please refer to the following table.

Step	Action
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.
3	• Apply 750 W (high) microwave irradiation for 1 min. • Cool rapidly by immediately adding 80 ml double dist. water (+20 to +25°C). • Transfer the slide(s) into **PBS** (+20 to +25°C). **DO NOT** perform a proteinase K treatment!
4	Immerse the slide(s) for 30 min at +15 to +25°C in **Tris-HCl, 0.1 M pH 7.5, containing 3% BSA and 20% normal bovine serum**.
5	Rinse the slide(s) twice with **PBS** at +15 to +25°C. Let excess fluid drain off.
6	Add 50 µl of **TUNEL reaction mixture** on the section and. ***Note***: For the negative control add 50 µl Label solution.
7	Incubate for 60 min at +37°C in a humidified atmosphere in the dark.
8	• Rinse slide(s) three times in **PBS** for 5 min each. • Samples can be analyzed in a drop of PBS under a fluorescence microscope at this state. Use an excitation wavelength in the range of 450–500 nm and detection in the range of 515–565 nm (green).

Additional equipment and solutions required

Washing buffer: PBS

- Humidified chamber
- Parafilm or coverslip
- DAB Substrate* or alternative POD substrate
- Mounting medium for light microscopy

Procedure

Please refer to the following table.

Step	Action
1	Dry area around sample.
2	Add 50 µl **Converter-POD** (vial 3) on sample. ***Note***: To ensure a homogeneous spread of Converter-POD across cell monolayer and to avoid evaporative loss, samples should be covered with parafilm or cover slip during incubation.
3	Incubate slide in a humidified chamber for 30 min at 37°C.
4	Rinse slide 3× with **PBS**.
5	Add 50 – 100 µl **DAB Substrate** or alternative POD substrates.
6	Incubate slide for 10 min at +15 to +25°C.
7	Rinse slide 3× with **PBS**.
8	Mount under glass coverslip (*e.g.,* with PBS/glycerol) and analyze under light microscope. Alternative: Samples can be counterstained prior to analysis by light microscope.

4. Appendix

4.1 Troubleshooting

This table describes various troubleshooting parameters.

Problem	Step/Reagent of Procedure	Possible cause	Recommendation
Nonspecific labeling	Embedding of tissue	UV-irradiation for polymerization of embedding material (*e.g.,* methacrylate) leads to DNA strand breaks	Try different embedding material or different polymerization reagent.
	Fixation	Acidic fixatives (*e.g.,* methacarn, Carnoy's fixative)	• Try 4% buffered paraformaldehyde. • Try formalin or glutaraldehyde.
	TUNEL reaction	TdT concentration too high	Reduce concentration of TdT by diluting it 1:2 up to 1:10 with TUNEL Dilution Buffer *.
	Converter solution	Endogenous POD activity	Block endogenous POD by immersing for 10 min in 3% H_2O_2 in methanol prior to cell permeabilisation.
		Non-specific binding of anti-fluorescein-POD	• Block with normal anti-sheep serum. • Block for 20 min with PBS containing 3% BSA. • Reduce concentration of converter solution to 50%.
	Nucleases	Some tissues (*e.g.,* smooth muscles) show DNA strand breaks very soon after tissue preparation	• Fix tissue immediately after organ preparation. • Perfuse fixative through liver vein.
		Some enzymes are still active	Block with a solution containing ddUTP and dATP.
High background	Fixation	Formalin fixation leads to a yellowish staining of cells containing melanin precursors	Try methanol for fixation but take into account that this might lead to reduced sensitivity.
	TUNEL reaction	Concentration of labeling mix is too high for mamma carcinoma	Reduce concentration of labeling mix to 50% by diluting with TUNEL Dilution Buffer*.
	Converter solution	Endogenous POD activity	Block endogenous POD by immersing for 10 min in 3% H_2O_2 in methanol prior to cell permeabilisation.
		Non-specific binding of anti-fluorescein-POD	• Block with normal anti-sheep serum. • Block for 20 min with PBS containing 3% BSA. • Reduce concentration of converter solution to 50%.
	Sample	Mycoplasma contamination	Mycoplasma detection Kit*.

Problem	Step/Reagent of Procedure	Possible cause	Recommendation
		Highly proliferating cells	Double staining *e.g.,*, with Annexin-V-Fluos*. ***Note***: Measuring via microplate reader not possible because of too high background.
Low labeling	Fixation	Ethanol and methanol can lead to low labeling (nucleosomes are not cross-linked with proteins during fixation and are lost during the procedure steps)	• Try 4% buffered paraformaldehyde. • Try formalin or glutaraldehyde.
		Extensive fixation leads to excessive crosslinking of proteins	• Reduce fixation time. • Try 2% buffered paraformaldehyde.
	Permeabilisation	Permeabilisation too short so that reagents can't reach their target molecules	• Increase incubation time. • Incubate at higher temperature (*e.g.,* +15 to +25°C). • Try Proteinase K , PCR Grade (concentration and time has to be optimized for each type of tissue). • Try 0.1 M sodium citrate at 70°C for 30 min.
	Paraffin-embedding	Accessibility for reagents is too low	• Treat tissue sections after dewaxing with Proteinase K, PCR Grade (concentration, time and temperature have to be optimized for each type of tissue). • Try microwave irradiation at 370 W (low) for 5 min in 200 ml 0.1 M Citrate buffer pH 6.0 (has to be optimized for each type of tissue).

Problem	Step/Reagent of Procedure	Possible cause	Recommendation
No signal on positive control	DNase treatment	Concentration of DNase is too low	• For cryosections apply 3 U/ml DNase I recombinant, grade I. • For paraffin-embedded tissue sections apply 1500 U/ml DNase I recombinant, grade I. • In general, use 1 U/ml DNase I recombinant, grade I, dissolved in 10 mM Tris-HCl pH 7.4 containing 10 mM NaCl, 5 mM $MnCl_2$, 0.1 mM $CaCl_2$, 25 mM KCl and incubate 30 min at +37°C. • Alternative buffer 50 mM Tris- HCl pH 7.5 containing 1 mM $MgCl_2$ and 1 mg/ml BSA.
Weak signals	Counterstaining	Not suitable dye	• Counterstaining with 5% methyl green in 0,1 M veronal acetate, pH 4.0 or Hematoxilin is possible (43). • Double-staining with propidium iodide is possible but only for detection of morphological cell changes.

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Apoptosis-specific physiological change	Detection mode/Product	Pack size	Cat. No.
DNA fragmentation	**Gel Electrophoresis**		
	Apoptotic DNA-Ladder Kit	20 tests	11 835 246 001
	***In situ* assay**		
	In Situ Cell Death Detection Kit, TMR red (also useable for FACS)	1 kit (50 tests)	12 156 792 910
	In Situ Cell Death Detection Kit, Fluorescein (also useable for FACS)	1 kit (50 tests)	11 684 795 910
	In Situ Cell Death Detection Kit, AP	1 kit (50 tests)	11 684 809 910
	In Situ Cell Death Detection Kit, POD	1 kit (50 tests)	11 684 817 910
	Single reagents for TUNEL and supporting reagents		
	TUNEL AP	70 tests (3.5 ml)	11 772 457 001
	TUNEL POD	70 tests (3.5 ml)	11 772 465 001
	TUNEL Enzyme	2× 50 µl (20 tests)	11 767 305 001
	TUNEL Label	3× 550 µl (30 tests)	11 767 291 910

*available from Roche Diagnostics

Changes to previous version

Editorial changes

Trademarks

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany