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

In Situ Cell Death Detection Kit, Fluorescein

Version: 20

Content Version: November 2020

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

Cat. No. 11 684 795 910 1 kit
50 tests

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	blue	*In Situ* Cell Death Detection Kit, Fluorescein, Enzyme Solution, 10x conc.	Terminal deoxynucleotidyl transferase from calf thymus *(EC 2.7.7.31)*, recombinant in *E. coli*, in storage buffer.	5 vials, 50 µl each
2	violet	*In Situ* Cell Death Detection Kit, Fluorescein, Label Solution, 1x conc.	Nucleotide mixture in reaction buffer. ⚠ ***Contains cacodylate and cobalt dichloride, see section, Safety Information.***	5 vials, 550 µl each

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the kit is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	blue	Enzyme Solution, 10x conc.	Store at −15 to −25°C.
2	violet	Label Solution, 1x conc.	

1.3. Additional Equipment and Reagent required

For preparation of sample material

ⓘ *See section,* **Preparation of sample material**.

Cell suspension

- Washing buffer: Phosphate buffered saline (PBS*)
- Fixation solution: 4% Paraformaldehyde in PBS*, pH 7.4, freshly prepared
- Permeabilization solution: 0.1% Triton X-100* in 0.1% sodium citrate, freshly prepared
- Shaker
- V-bottomed 96-well microplate
  ⓘ *Use of a V-bottomed 96-well microplate minimizes cell loss during fixation, permeabilization, and labeling, and allows simultaneous preparation of multiple samples.*

Adherent cells, cell smears, cytospin preparations, and cryopreserved tissue

- Washing buffer: Phosphate buffered saline (PBS*)
- Fixation solution: 4% Paraformaldehyde in PBS*, pH 7.4, freshly prepared
- Permeabilization solution: 0.1% Triton X-100* in 0.1% sodium citrate, freshly prepared

Treatment of paraffin-embedded tissue

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

Alternative treatments for paraffin-embedded tissue

- Permeabilization solution: 0.1% Triton X-100*, 0.1% sodium citrate, freshly prepared
- Pepsin*: 0.25% to 0.5% in HCl, pH 2, or Trypsin*, 0.01 N HCl, nuclease free
- 0.1 M citrate buffer, pH 6 for microwave irradiation

For labeling protocol

See section, **Labeling protocol**.

Positive control

- Nuclease S7, Micrococcal nuclease* or DNase I recombinant, grade I*

Cell suspension

- Washing buffer: PBS*
- Humidified chamber

Adherent cells, cell smears, cytospin preparations, and tissue sections

- Washing buffer: PBS*
- Parafilm or coverslips
- Humidified chamber

Difficult tissue

- 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

1.4. Application

The *In Situ* Cell Death Detection Kit, Fluorescein is designed as a precise, fast and simple, nonradioactive technique to detect and quantify apoptotic cell death at single cell level in cells and tissues. The kit can be used in many different assay systems:

- Detection of individual apoptotic cells in frozen and formalin-fixed tissue sections in basic research.
- 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.

1.5. Preparation Time

Assay Time

1 to 2 hours, excluding culture, fixation and permeabilization of cells, and preparation of tissue sections.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The *In Situ* Cell Death Detection Kit, Fluorescein can be used with a variety of sample materials:
- Cell suspensions from permanent cell lines, lymphocytes and leukemic cells from peripheral blood, thymocytes, bone marrow cells, and fine-needle biopsies.
- Cytospins and cell smear preparations.
- Adherent cells cultured on chamber slides.
- Frozen or formalin-fixed, paraffin-embedded tissue sections.

Control Reactions

Preparation of controls for labeling protocol

For additional information, see section, **Additional reagents and required materials**.
Include two negative controls and one positive control in each experimental setup.

Control	Preparation
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 Nuclease S7* or DNase I recombinant, grade I* (3,000 U/ml to 3 U/ml in 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mg/ml BSA) for 10 minutes at +15 to +25°C to induce DNA strand breaks prior to labeling procedures.

General Considerations

Test interference

False-negative results

DNA cleavage can be absent or incomplete in some forms of apoptotic cell death. Steric hindrance such as extracellular matrix components can prevent access of TdT to DNA strand breaks. In either case, false-negative results are obtained.

False-positive results

Extensive DNA fragmentation may occur in certain forms of necrosis. 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 verify apoptotic mode of cell death, carefully examine the morphology of respective cells. 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.

Safety Information

Precautions

The Label Solution (Vial 2) contains cacodylate, toxic if inhaled and swallowed, and cobalt dichloride, which may cause cancer when inhaled. Avoid exposure and follow special instructions before use:

- Do not eat, drink, or smoke. After contact with skin, wash immediately with plenty of water. In case of accident or if you feel ill, 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.

For customers in the European Economic Area

Contains SVHC: octyl/nonylphenol ethoxylates. For use in research and under controlled conditions only – acc. to Art. 56.3 and 3.23 REACH Regulation.

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink, or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats, and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Preparation of TUNEL reaction mixture

The TUNEL reaction mixture is used in the section, **Labeling Protocol**.

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.

⚠ ***Prepare the TUNEL reaction mixture immediately before use; do not store. Keep TUNEL reaction mixture on ice until use.***

1. Remove 100 µl Label Solution (Vial 2) for two negative controls.

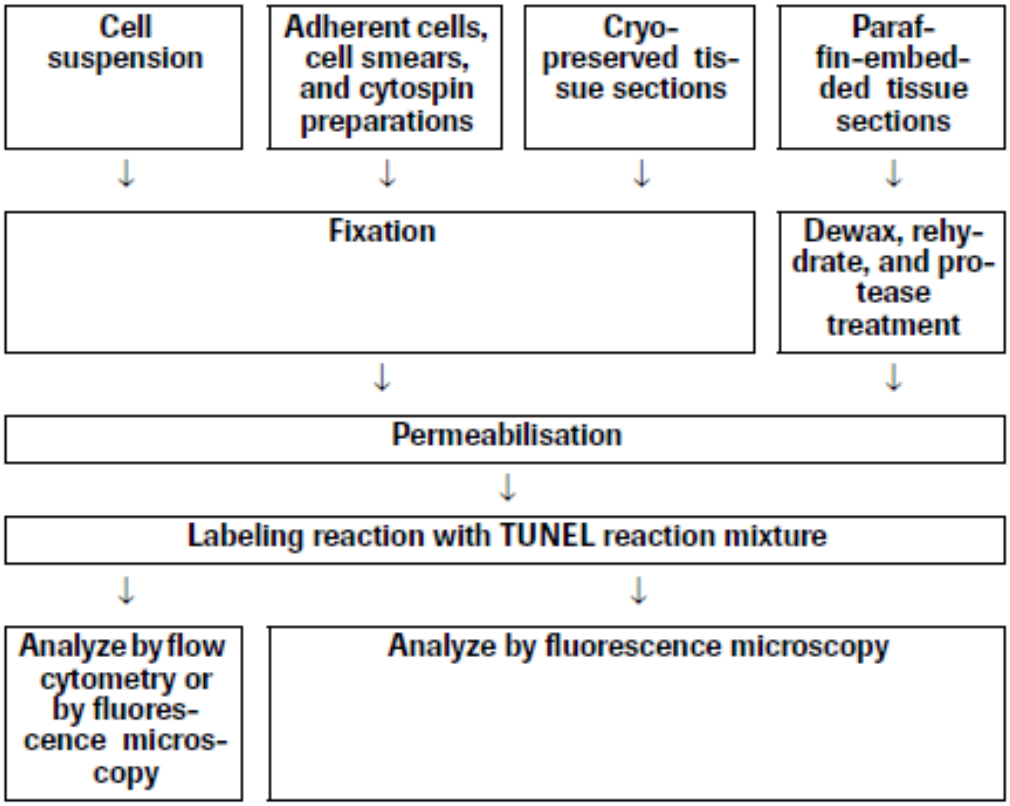
2. Add total volume of 50 µl of Enzyme Solution (Vial 1) to the remaining 450 µl Label Solution in Vial 2 to obtain 500 µl TUNEL reaction mixture.

3. Mix well to equilibrate components.

2.2. Protocols

Assay overview

The overview described below uses fluorescein-dUTP to easily and quickly label DNA strand breaks, allowing the direct detection of DNA fragmentation by flow cytometry or fluorescence microscopy.



Preparation of sample material

Cell suspensions

For dual-parameter flow cytometry with fluorescein-conjugated antibodies, incubate the cells prior to fixation with the cell surface marker.

The following steps describe the fixation and permeabilization of cells.
⚠ ***Fix and permeabilize two additional cell samples for the negative and positive labeling controls.***

1. Wash test sample 3 times in PBS and adjust to 2 × 10^7 cells/ml.

2. Transfer 100 µl/well cell suspension into a V-bottomed 96-well microplate.

3. Add 100 µl/well of a freshly prepared Fixation solution to cell suspension.
   Final concentration 2% PFA.

4. Resuspend completely and incubate 60 minutes at +15 to +25°C.
   To avoid extensive clumping of cells, incubate microplate on a shaker during fixation.

5. Centrifuge microplate at 300 × *g* for 10 minutes and remove fixative by flicking off or suction.

6. Wash cells once with 200 µl/well PBS.

7. Centrifuge microplate at 300 × *g* for 10 minutes and remove PBS by flicking off or suction.

8. Resuspend cells in 100 µl/well Permeabilization solution for 2 minutes on ice at +2 to +8°C.

9. Proceed as described in section, **Labeling protocol**.

Adherent cells, cell smears, and cytospin preparations

The following steps describe the fixation and permeabilization of cells.
⚠ ***Fix and permeabilize two additional cell samples for the negative and positive labeling controls.***

1. Fix air-dried cell samples with the freshly prepared Fixation solution for 1 hour at +15 to +25°C.

2. Rinse slides with PBS.

3. Incubate in Permeabilization solution for 2 minutes on ice at +2 to +8°C.

4. Proceed as described in section, **Labeling protocol**.

Treatment of paraffin-embedded tissue

Tissue sections can be pretreated in 4 different ways. If you use Proteinase K*, the concentration, incubation time, and temperature must be optimized for each type of tissue.
⚠ ***Only use Proteinase K that is tested for the absence of nucleases to avoid false-positive results.***

Perform the following steps for the pretreatment of paraffin-embedded tissue with Proteinase K and 3 alternative procedures (Step 2).
⚠ ***Add additional tissue sections for the negative and positive labeling controls.***

1. Dewax and rehydrate tissue sections according to standard protocols, such as by heating at +60°C, followed by washing in xylene and rehydration through a graded series of ethanol and double-distilled water.

2. Incubate tissue sections for 15 to 30 minutes at +21 to +37°C with Proteinase K working solution.
– If the inactivation of endogenous nucleases with Proteinase K does not work with your samples, use one of the following alternative treatments:

Alternative	Treatment
Permeabilization solution	Incubate slides for 8 minutes.
Pepsin or Trypsin	15 to 60 minutes at +37°C.
Microwave irradiation	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 minutes.

3. Rinse slide(s) twice with PBS.

4. Proceed as described in section, **Labeling protocol**.

Treatment of cryopreserved tissue

The following steps describe the pretreatment of cryopreserved tissue.
⚠ ***Fix and permeabilize two additional samples for the negative and positive labeling controls.***

1. Fix tissue section with Fixation solution for 20 minutes at +15 to +25°C.

2. Wash 30 minutes with PBS.
   ⚠ ***For storage, dehydrate fixed tissue sections 2 minutes in absolute ethanol and store at −15 to −25°C.***

3. Incubate slides in Permeabilization solution for 2 minutes on ice at +2 to +8°C.

4. Proceed as described in section, **Labeling protocol**.

Labeling protocol

Cell suspensions

1. Wash cells twice with 200 µl/well PBS.

2. Resuspend in 50 µl/well TUNEL reaction mixture.
   i *For the negative control, add 50 µl Label Solution each.*

3. Add lid and incubate for 60 minutes at +37°C in a humidified atmosphere in the dark.

4. Wash samples twice in PBS.

5. Transfer cells into a tube to a final volume of 250 to 500 µl in PBS.

6. Analyze samples directly under a fluorescence microscope or embedded with antifade prior to analysis.
   – Use an excitation wavelength of 450 to 500 nm and detection wavelength of 515 to 565 nm (green).

Adherent cells, cell smears, cytospin preparations, and tissues

1. Rinse slides twice with PBS.

2. Dry area around sample.

3. Add 50 µl TUNEL reaction mixture on sample.
   i *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, cover samples with parafilm or a coverslip during incubation.*

4. Add lid and incubate for 60 minutes at +37°C in a humidified atmosphere in the dark.

5. Rinse slide(s) 3 times with PBS.
   – Analyze samples in a drop of PBS under a fluorescence microscope. Use an excitation wavelength of 450 to 500 nm and detection wavelength of 515 to 565 nm (green).

Difficult tissue

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 minute.
   – Cool rapidly by immediately adding 80 ml double-distilled 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 minutes at +15 to +25°C in 0.1 M, pH 7.5 Tris-HCl, containing 3% BSA and 20% normal bovine serum.

5. Rinse the slide(s) twice with PBS at +15 to +25°C.
   – Drain off excess fluid.

6. Add 50 µl of TUNEL reaction mixture on the section.
   For the negative control, add 50 µl Label Solution.

7. Incubate for 60 minutes at +37°C in a humidified atmosphere in the dark.

8. Rinse slide(s) three times in PBS for 5 minutes each.
   – Analyze samples in a drop of PBS under a fluorescence microscope. Use an excitation wavelength of 450 to 500 nm and detection wavelength of 515 to 565 nm (green).

2.3. Parameters

Sensitivity

The *In Situ* Cell Death Detection Kit, Fluorescein detects apoptotic cell death at the single-cell level via fluorescence microscopy and at cell populations via FACS analysis at very early stages.

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. Results

Analysis of camptothecin-induced apoptosis by flow cytometry

1. Incubate HL-60 cells at a cell density of 5 × 10^5 cells/ml in the presence of camptothecin (2 µg/ml, 3 hours at +37°C, 5% CO_2 , 90% humidity) to induce apoptosis (Fig.1).

2. As a control for a non-apoptotic cell population, incubate an aliquot of the cells in medium without camptothecin.

3. Harvest cells and proceed as described in section, **Labeling protocol, Cell suspensions**.

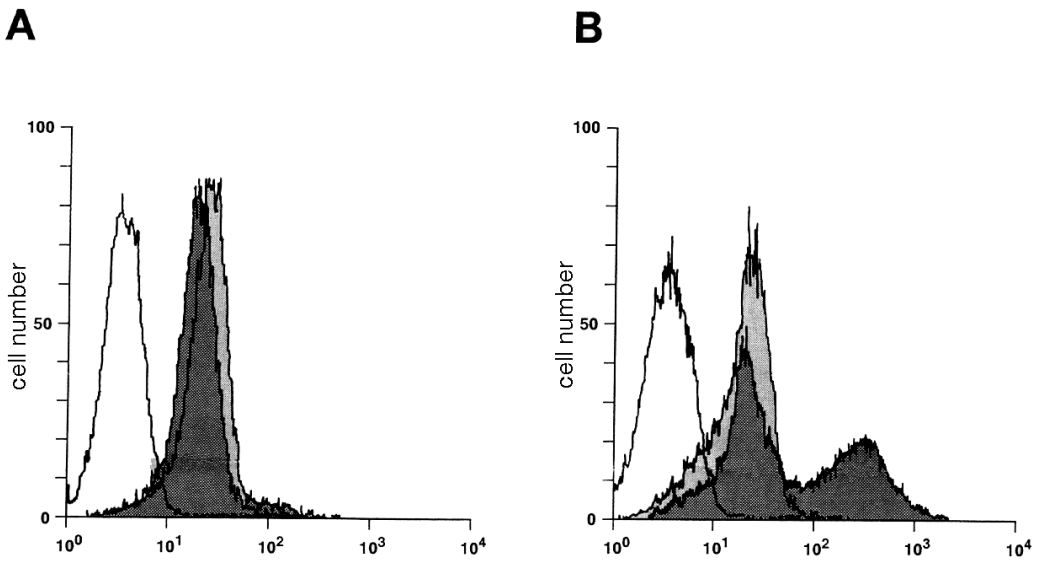


Fig. 1: HL-60 cells were cultured as described above. Subsequently, apoptotic cells were labeled as described in section,Labeling protocol, Cell suspensions.
A: Cells cultured in the absence of camptothecin.
B: Cells cultured in the presence of camptothecin (2 µg/ml, 3 hours).
White: Control for autofluorescence of cells, without incubation with Label or Enzyme Solution.
Grey: Negative control, incubated with Label Solution, in the absence of terminal transferase.
Black: Test sample, incubated with TUNEL reaction mixture.

4. Troubleshooting

Observation	Possible cause	Recommendation
Nonspecific labeling	UV-irradiation for polymerization of embedding material, such as methacrylate, leads to DNA strand breaks.	Check tissue-embedding protocol.
		Use a different embedding material or different polymerization reagent.
	Acidic fixatives, such as methacarn or Carnoy's fixative.	Check fixation method.
		Use formalin, glutaraldehyde, or 4% buffered paraformaldehyde for fixation.
	TdT concentration too high.	Check TUNEL reaction.
		Reduce TdT concentration by diluting 1:2 to 1:3 with TUNEL Dilution Buffer*.
	Some tissues, such as smooth muscles, show DNA strand breaks immediately after tissue preparation.	Check for presence of nucleases and polymerases.
		Fix tissue immediately after organ preparation.
		Perfuse fixative through liver vein.
	Some enzymes are still active.	Check for presence of nucleases and polymerases.
		Block with a solution containing ddUTP and dATP.
High background present.	Mycoplasma contamination present.	Check sample using the Mycoplasma PCR ELISA*.
	Highly proliferating cells.	Check sample.
		Double stain with Annexin-V-FLUOS*. *Measuring using a microplate reader is not possible because of too high background.*
	Erythrocytes have high autofluorescence due to hemoglobin.	Check sample.
		Use dUTP-rhodamine.
	Formalin fixation leads to a yellowish staining of cells containing melanin precursors.	Check fixation method.
		Use methanol for fixation but take into account that this might lead to reduced sensitivity.
	Concentration of labeling mix is too high for mamma carcinoma.	Check TUNEL reaction.
		Reduce concentration of labeling mix to 50% by diluting with TUNEL Dilution Buffer*.

Low labeling	Ethanol and methanol can lead to low labeling because nucleosomes are not crosslinked with proteins during fixation and are lost during the protocol steps.	Check fixation method.
		Use formalin, glutaraldehyde, or 4% buffered paraformaldehyde for fixation.
	Extensive fixation leads to excessive crosslinking of proteins.	Check fixation method.
		Reduce fixation time.
		Use 2% buffered paraformaldehyde.
	Permeabilization too short so that reagents cannot reach their target molecules.	Check permeabilization step.
		Increase incubation time.
		Incubate at higher temperature, for example, +15 to +25°C.
		Use Proteinase K* (optimize concentration and time for each type of tissue).
		Use 0.1 M sodium citrate at +70°C for 30 minutes.
	Fluorescence lasts 10 minutes under bright light (bleaching).	Keep samples in the dark after TUNEL reaction for later inspections.
	Accessibility for reagents is too low.	Check paraffin-embedding step.
		Treat tissue sections after dewaxing with Proteinase K* (optimize concentration and time for each type of tissue).
		Use microwave irradiation at 370 W (low) for 5 minutes in 200 ml 0.1 M citrate buffer, pH 6.0 (optimize for each type of tissue).
No signal for positive control.	Concentration of DNase is too low.	Check DNase treatment method.
		For cryosections, add 3 U/ml DNase I recombinant, grade I*.
		For paraffin-embedded tissue sections, add 1,500 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 minutes at +37°C.
		Use an alternative buffer of 50 mM Tris-HCl, pH 7.5, containing 1 mM $MgCl_2$ and 1 mg/ml BSA*.
Counterstaining diminishes TUNEL staining.	Propidium iodide quenches light emitted by fluorescein via energy transfer.	Check DNA stain.
		Use 0.5 µg/ml propidium iodide.
		Counterstain the cytoplasm with sulforhodamine.
		Try a dye that exhibits far-red fluorescence to get a clear separation of the emission spectrum like TO-PRO-3.
Equivocal signals not obtained.	Earlier stage of apoptosis than stage detected by TUNEL reaction.	Check double-staining step.
		For additional measurement of apoptosis, use M30 CytoDEATH* or Annexin-V-FLUOS*.

Problems with interpretation of results.	Too much overlap between the different peaks due to a late analysis of the apoptotic reactions, and a mix of apoptotic cells and apoptotic bodies.	Check FACS Analysis.
		Reduce the duration/strength of the apoptosis treatment to be able to identify 2 to 3 different clusters/peaks: ▪ Debris and apoptotic bodies ▪ Apoptotic cells ▪ Viable cells
		Optimize the washing step and control the buffers used.
		Use other surface markers and adapt gating strategy to refine the definition of the different steps of the apoptosis, and reduce and avoid the peak related to the debris/apoptotic bodies.
	No signal for apoptosis.	Check FACS Analysis.
		Optimize incubation time for cell line and inducing agents.

5. Additional Information on this Product

5.1. 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 (Fig. 2).

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. Fluorescein labels incorporated in nucleotide polymers are detected and quantified by fluorescence microscopy or flow cytometry.

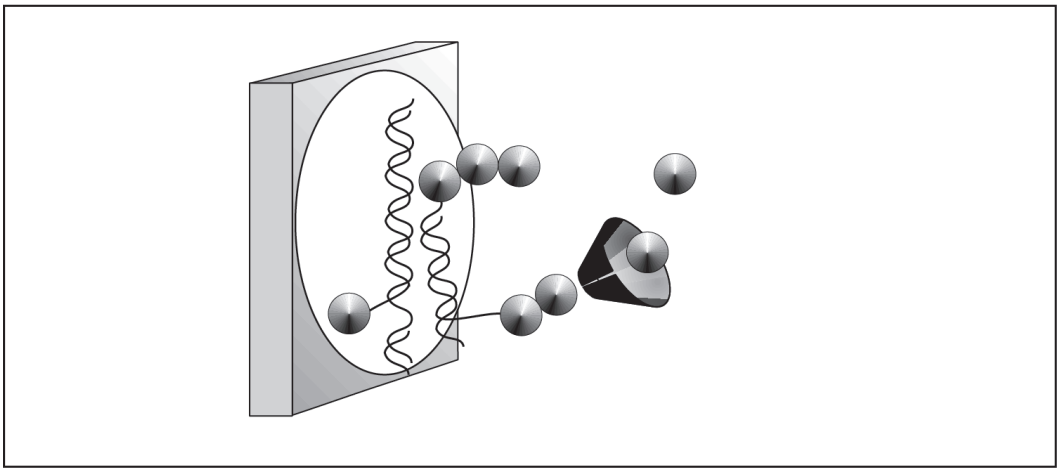


Fig. 2: DNA of fixed cells labeled by the addition of fluorescein dUTP at strand breaks by terminal transferase.

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. 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. However, very rare exceptions have been described where morphological features of apoptosis are not accompanied with oligonucleosomal DNA cleavage.

Apoptosis

Apoptosis is essential in many physiological processes, including maturation and effector mechanisms of the immune system, embryonic development of tissue, organs and limbs, development of the nervous system, and hormone-dependent tissue remodeling. Inappropriate regulation of apoptosis may play an important role in many pathological conditions, such as ischemia, stroke, heart disease, cancer, AIDS, autoimmunity, hepatotoxicity, and degenerative diseases of the central nervous system.
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.

Identification of apoptosis

Several methods have been described to identify apoptotic cells. 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, cannot 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 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) or TUNEL (TdT-mediated dUTP nick end labeling) 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.
- Kinetics of nucleotide incorporation is very rapid with TUNEL compared to the ISNT.
- 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.

5.2. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.
New information added related to the REACH Annex XIV.
Update to include new safety Information to ensure handling according controlled conditions.

6.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Pepsin	1 g, *Not available in US*	10 108 057 001
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Trypsin recombinant, Proteomics Grade	4 x 25 µg	03 708 985 001
	4 x 100 µg	03 708 969 001
Annexin-V-FLUOS	500 µl, 250 tests	11 828 681 001
TUNEL Dilution Buffer	2 x 10 ml	11 966 006 001
Nuclease S7	15,000 U	10 107 921 001
Bovine Serum Albumin	20 mg, 1 ml	10 711 454 001
Proteinase K, recombinant, PCR Grade	25 mg	03 115 836 001
	100 mg	03 115 879 001
	2 x 250 mg	03 115 801 001
	4 x 250 mg	03 115 852 001
Triton X-100	100 ml	10 789 704 001

6.4. Trademarks

All product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

6.6. Regulatory Disclaimer

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

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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