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

TUNEL Label Mix

Version: 12
Content Version: December 2020

Nucleotide mix, containing fluorescein-dUTP and dNTP

Cat. No. 11 767 291 910 3 x 550 µl
30 tests

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	purple	TUNEL Label Mix	▪ Solution of Fluorescein-dUTP and unlabeled dNTP in optimized concentrations and ratios. ▪ Reaction buffer (200 mM potassium cacodylate, 25 mM Tris-HCl, 1 mM $CoCl_2$, 0.25 mg/ml bovine serum albumin, pH 6.6). ▪ Optimized for TUNEL Enzyme (TdT).	3 bottles, 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	purple	TUNEL Label Mix	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For preparation of sample material

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

Cell suspensions

- 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
  These plates minimize cell loss during fixation, permeabilization and labeling, and 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
- Blocking solution: 3% H_2O_2 in methanol (blocks endogenous peroxidase for the detection with TUNEL POD*)

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**.

Preparation of TUNEL reaction mixture

- TUNEL Enzyme*

Positive controls

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

Cell suspensions

- 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

For signal conversion (optional)

See section, **Signal conversion and analysis**.

- Washing buffer: PBS*
- TUNEL AP*: Anti-fluorescein antibody, Fab fragments from sheep, conjugated with alkaline phosphatase
- TUNEL POD*: Anti-fluorescein antibody, Fab fragments from sheep, conjugated with horseradish peroxidase
- Substrate solution for TUNEL AP: NBT/BCIP* or Fast Red
- Substrate solution for TUNEL POD: Dab Substrate or alternative POD substrate
- Mounting medium for light microscopy
- Humidified chamber
- Parafilm or coverslip

1.4. Application

The TUNEL Label Mix is used in combination with the TUNEL Enzyme* to prepare the TUNEL reaction mixture. The TUNEL reaction mixture is used to label DNA strand breaks for detecting and quantitating apoptotic cell death at single-cell level in cells and tissues *in situ*.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The TUNEL Label Mix can be used with the following sample materials:
- Cells in suspension.
- Cytospin and cell smear preparations.
- Adherent cells cultured on chamber slides.
- Frozen or formalin-fixed, plastic- or 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 TUNEL 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.

Safety Information

Precautions

Reaction buffer 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.

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

1. For 1 test, add 5 µl of TUNEL Enzyme solution to 45 µl TUNEL Label solution to obtain 50 µl TUNEL reaction mixture.

2. Mix well to equilibrate components.

2.2. Protocols

Preparation of sample material

Cell suspensions

Prelabeling
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% paraformaldehyde).

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

he 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 with Blocking solution for 10 minutes at +15 to +25°C; then rinse slides with PBS.
   ℹ *This is an optional step; only necessary for the detection with TUNEL POD.*

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

5. 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 with Blocking solution for 10 minutes at +15 to +25°C; then rinse slides with PBS.
   ℹ *This is an optional step; only necessary for the detection with TUNEL POD.*

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

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

Labeling protocol

Cell suspensions

1. Wash cells 2 times with PBS (200 µl/well).

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

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

4. Wash cells 3 times in PBS (200 µl/well).
   – Analyze cells by fluorescence microscopy. Use an excitation wavelength in the range of 450 to 500 nm and detection wavelength of 515 to 565 nm (green).

   ℹ *For analysis by flow cytometry, dilute cells approximately 1:10 in PBS.*

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 controls, 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 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 in the range 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 Tris-HCl, pH 7.5, 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.
   i 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).

Signal conversion and analysis

Using TUNEL AP or TUNEL POD, the fluorescent label may be converted into a colorimetric signal, allowing the samples to be analyzed by light microscopy. If preparations will be analyzed by light microscopy using TUNEL AP or TUNEL POD as a secondary detection system, any precipitating substrate suitable for immunohistochemistry may be used.

1. Dry area around sample.

2. Add 50 µl TUNEL AP or TUNEL POD on sample.
   i To ensure a homogeneous spread of Converter-AP or Converter-POD across cell monolayer and to avoid evaporative loss, cover samples with parafilm or a coverslip during incubation.

3. Incubate slide in a humidified chamber for 30 minutes at +37°C.

4. Rinse slide 3 times with PBS.

5. Add 50 to 100 µl Substrate solution.

6. Incubate slide for 10 minutes at +15 to +25°C.

7. Rinse slide 3 times with PBS.

8. Mount under a glass coverslip, using for example, PBS/glycerol, and analyze under a light microscope.

i As an alternative, samples can be counterstained prior to analysis by light microscope.

3. Additional Information on this Product

3.1. Test Principle

The main advantage of this simple and rapid procedure is the use of fluorescein-dUTP to label DNA strand breaks. This enables the detection of DNA degradation by fluorescence microscopy or flow cytometry directly after the TUNEL reaction. It has been shown that this direct detection of DNA strand breaks is as powerful as indirect detection methods, such as using DIG-dUTP and a fluorescein-labeled anti-DIG antibody.

Identification of apoptosis

DNA degradation is a key biochemical event of apoptosis, resulting in the cleavage of nuclear DNA into oligonucleosome-sized fragments. This process is widely used for detecting 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 DNA strand breaks which occur early during apoptosis. 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.

4. Supplementary Information

4.1. 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.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. 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
NBT/BCIP Stock Solution	8 ml	11 681 451 001
TUNEL POD	3.5 ml, 70 tests	11 772 465 001
TUNEL Enzyme	2 x 50 µl, 20 tests	11 767 305 001
TUNEL AP	3.5 ml, 70 tests	11 772 457 001
DNase I recombinant, RNase-free	10,000 U, 10 U/µl	04 716 728 001
Nuclease S7	15,000 U	10 107 921 001
DAB Substrate	1 pack	11 718 096 001
Triton X-100	100 ml	10 789 704 001
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

4.4. Trademarks

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

4.5. License Disclaimer

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

4.6. Regulatory Disclaimer

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

4.7. Safety Data Sheet

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

4.8. 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.

1220.11784307001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany