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

TUNEL Dilution Buffer

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For dilutions of the terminal transferase within the TUNEL reaction mix for detection of apoptosis (programmed cell death) *in situ*

Cat. No. 11 966 006 001 2 x 10 ml

Store the product at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	red	TUNEL Dilution Buffer	Buffer solution containing 30 mM Tris/HCl, 140 mM sodium cacodylate, and 1 mM $CoCl_2$.	2 bottles, 10 ml each

1.2. Storage and Stability

Storage Conditions (Product)

Shipped on dry ice.
When stored at +2 to +8°C, the product is stable through the expiration date printed on the label.

Vial / Bottle	Cap	Label	Storage
1	red	TUNEL Dilution Buffer	Store at +2 to +8°C.

1.3. Application

The TUNEL Dilution Buffer can be used:
- In combination with the *In Situ* Cell Death Detection Kits*.
- To dilute TdT for the TUNEL reaction in special applications.
- To label DNA strand breaks for detecting and quantitating apoptotic cell death at single-cell level in cells and tissues.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

The TUNEL Dilution Buffer 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.

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, **Protocol**.

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

1. Mix 5 µl of the TUNEL Enzyme* dilution with 45 µl of the TUNEL Label Mix* to obtain 50 µl TUNEL reaction mixture.

2. Mix well to equilibrate components.

2.2. Protocols

Use your *In Situ* Cell Death Detection Kit as described in its Instructions for Use. In parallel, apply dilutions of TdT to the tissue sections for overcoming false-positive labeling.

Preparation of the dilutions of the TUNEL Enzyme (TdT)

1. Prepare the dilutions as described in the table below.
   Prepare dilutions immediately before use; do not store.

TdT Dilution	TdT	TUNEL Dilution Buffer [µl]	Total Volume of TdT Dilution [µl]
Undiluted	5 µl of TUNEL Enzyme	–	5
1:2	5 µl of TUNEL Enzyme	5	10
1:3	5 µl of TUNEL Enzyme	10	15
1:5	5 µl of TUNEL Enzyme	20	25
1:10	5 µl out of 1:5	5	10
1:50	5 µl out of 1:10	20	25
1:100	10 µl out of 1:50	10	20

2. Apply the 50 µl of the TUNEL reaction mixture onto the tissue section.
   See section, Working Solution.

3. Proceed as describe in the Instructions for Use of the *In Situ* Cell Death Detection Kit.

Evaluation of the staining

Evaluate your dilutions by microscopy. The specificity of the staining will increase with dilution if an over-staining is observed with the undiluted kit components. At a certain dilution, the TUNEL reaction is optimal and the signal will diminish by further dilution of TdT.

3. Additional Information on this Product

3.1. Test Principle

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.

For certain tissue sections (mostly in proliferating tissue), the concentration of TdT in our *In Situ* Cell Death Detection Kits has to be adjusted because Okazaki fragments or other criteria existing inside the cells can influence the labeling. To overcome this problem of overstaining, a dilution of the TdT component of the *In Situ* Cell Death Detection Kit* is helpful.

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		
In Situ Cell Death Detection Kit, Fluorescein	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
TUNEL Label Mix	3 x 550 µl, 30 tests	11 767 291 910
TUNEL Enzyme	2 x 50 µl, 20 tests	11 767 305 001

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.

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