

EdUTP-488 TUNEL Apoptosis Detection Kit

E1456515

Storage Store at -20 °C. Protect from light. For detailed storage conditions, please refer to the product components.

Shipping Ultra-low temperature transportation. Upon receipt, store immediately at the recommended storage conditions.

Introduction

This kit is an apoptosis detection assay based on click chemistry-enhanced TUNEL technology, designed for high-sensitivity detection of DNA fragmentation in apoptotic cells.

During apoptosis, endogenous endonucleases are activated and cleave genomic DNA at the internucleosomal linker regions into 180-200 bp fragments, generating abundant 3'-OH termini.

This kit utilizes terminal deoxynucleotidyl transferase (TdT) to catalyze the incorporation of EdUTP (5-ethynyl-dUTP, an alkyne-modified dUTP) onto the 3'-OH ends of fragmented DNA. Subsequently, in the presence of a monovalent copper ion catalyst, the alkyne group in EdUTP undergoes a highly specific click reaction with Azide 488 (an AF488 fluorescent probe conjugated to an azide moiety), thereby labeling the DNA break sites in apoptotic cells with green fluorescence.

Components

T1456515	Components	20 T	50 T	Storage	Quantity Per Test
T1456515A	Recombinant TdT Enzyme	20 µL	50 µL	-20°C.	1 µL
T1456515B	5-EdUTP	40 µL	100 µL	-20°C.	2 µL
T1456515C	Reaction Buffer(5×)	200 µL	500 µL	-20°C.	10 µL
T1456515D	Azide 488	20 µL	50 µL	-20°C. Store in the dark	2 µL
T1456515E	CuSO ₄	1 mL	2.5 mL	-20°C.	2 µL
T1456515F	Copper Protectant	200µL	500µL	-20°C.	5 µL
T1456515G	Click Additive	20mg	50mg	-20°C.	10 µL
T1456515H	Hoechst 33342(1000×)	20 µL	50 µL	-20°C. Store in the dark	1 µL

Protocol

1. Experimental Materials (Self-Provided)

- 1) 1×PBS (pH 7.4).
- 2) 4% Paraformaldehyde (fixative solution, prepared in PBS).
- 3) Permeabilization solution: 0.1-0.2% Triton X-100(prepared in PBS) or 20 µg/mL Proteinase K.
- 4) DNase I (for positive control treatment).
- 5) Anti-fluorescence fading mounting medium.
- 6) Coverslips, glass slides, and humidified chamber.
- 7) Distilled water or deionized water.
- 8) Xylene and graded ethanol (for paraffin-embedded sections).

2. Cell Smears/Cytospin Preparations

- 1) Wash: Wash the cells once with PBS.
- 2) Fixation: Add an appropriate volume of 4% paraformaldehyde and fix at room temperature for 30 minutes or at 4°C for 1-2 hours.
- 3) Wash three times with PBS, 5 minutes each.
- 4) Permeabilization: Add an appropriate volume of 0.1% Triton X-100 and permeabilize at room temperature for 15 minutes. Wash twice with PBS.

3. Paraffin-Embedded Tissue Sections

- 1) Deparaffinization and Rehydration: Deparaffinize in xylene for 5-10 minutes. Transfer to fresh xylene and repeat for another 5-10 minutes. Then incubate in absolute ethanol for 5 minutes, 90% ethanol for 2 minutes, 70% ethanol for 2 minutes, and distilled water for 2 minutes.
- 2) Permeabilization: Dilute Proteinase K (P274341, 2 mg/mL) with PBS to a final concentration of 20 µg/mL. Add 100 µL of this solution to each sample to cover the tissue, and incubate at 37°C for 15-30 minutes.

Note: Prolonged incubation may increase the risk of tissue detachment from slides during subsequent washing steps, whereas insufficient incubation may result in inadequate permeabilization and compromised labeling efficiency. The optimal incubation temperature and duration should be empirically determined for different tissue types.

- 3) Wash: Wash three times with PBS, 5 minutes each.

Note: It is critical to thoroughly remove Proteinase K at this step, as residual enzyme activity will severely interfere with subsequent labeling reactions.

4. Frozen Tissue Sections

- 1) Fixation: Immerse the section slides in 4% paraformaldehyde and fix at room temperature for 30 minutes. Gently rinse three times with PBS, 5 minutes each.
- 2) Permeabilization: Dilute Proteinase K (2 mg/mL) with PBS to a final concentration of 20 µg/mL. Add 100 µL of this solution to each sample to cover the tissue, and incubate at 37°C for 10 minutes.

Note: Prolonged incubation may increase the risk of tissue detachment from slides during

subsequent washing steps, whereas insufficient incubation may result in inadequate permeabilization and compromised labeling efficiency. The optimal incubation temperature and duration should be empirically determined for different tissue types.

- 3) Rinse the sections three times with PBS, 5 minutes each. Use a hydrophobic barrier pen to delineate the sample contours for downstream labeling. Keep the processed sections in a humidified chamber to maintain moisture.

Note: If the hydrophobic barrier ring is found to be damaged during subsequent experimental procedures, it should be promptly reapplied.

5. Positive Control (Optional)

- 1) Dilute DNase I (10 U/ μ L) with DNase I dilution buffer to a final concentration of 100 U/mL.
- 2) Add 50 μ L DNase I solution onto the pretreated sample, and incubate at room temperature for 10 minutes.
- 3) Remove DNase I solution and wash the sample twice with PBS.

6. TUNEL Reaction Labeling Master Mix(Prepare Fresh Immediately Before Use)

- 1) Equilibration: Dilute the 5 $\times$ Reaction Buffer with ddH₂O to prepare 1 $\times$ TdT Reaction Buffer. Add 100 μ L of the 1 $\times$ TdT Reaction Buffer to each sample, and incubate in a humidified chamber at 37°C for 10-30 minutes to allow equilibration.
- 2) Prepare the labeling working solution according to the proportions provided below. All operations must be performed on ice, protected from light, and thoroughly mixed. Calculate the required volume for each sample (50 μ L system) as follows:

Components of the TUNEL Labeling Working Solution	One sample	Five samples	Ten samples
Recombinant TdT Enzyme	1 μ L	5 μ L	10 μ L
5-EdUTP	2 μ L	10 μ L	20 μ L
Reaction Buffer(5 $\times$)	10 μ L	50 μ L	100 μ L
ddH ₂ O	37 μ L	185 μ L	370 μ L
Total Volume	50 μ L	250 μ L	500 μ L

Note: The Reaction Buffer and Recombinant TdT Enzyme must be completely dissolved and mixed thoroughly before use.

- 3) Labeling Reaction: Aspirate the equilibration buffer (taking care not to allow the slides to dry out). Add 50 μ L of the labeling working solution to each sample, cover with a coverslip or sealing film, and incubate in a humidified chamber at 37°C in the dark for 60 minutes.
- 4) Termination Reaction: Wash 3 times with PBS, 5 minutes each wash.

7. Click Reaction (Fluorescence Labeling)

- 1) Preparation of Click Additive Solution: For the 20T kit, dissolve the provided 1 vial of Click Additive in 0.36 mL of ultrapure water, and mix thoroughly until completely dissolved to obtain the Click Additive Solution. For the 50T kit, dissolve the provided 1 vial of Click Additive in 0.9 mL of ultrapure water, and mix thoroughly until completely dissolved to obtain the Click Additive Solution. After preparation, the solution may be appropriately

aliquoted and stored at -20°C.

- 2) Prepare the Click Reaction Working Solution according to the proportions below (prepare fresh immediately before use). The required volume is calculated per sample (50 µL system):

Components of the Click Reaction Working Solution	One sample	Five samples	Ten samples
Azide 488	1 µL	5 µL	10 µL
CuSO ₄	2 µL	10 µL	20 µL
Copper Protectant	5 µL	10 µL	50 µL
Click Additive Solution	10 µL	50 µL	100 µL
ddH ₂ O	33 µL	165 µL	330 µL
Total Volume	50 µL	250 µL	500 µL

Note: 50 µL of the biotin labeling solution is suitable for smears, sections, or one well of a 96-well, 48-well, 24-well, or 12-well plate. For one well of a 6-well plate, 100 µL of the biotin labeling solution is recommended. During incubation, take precautions to prevent evaporation of the working solution.

- 3) Add 50 µL of the Click Reaction Working Solution to each sample and incubate at room temperature in the dark for 30 minutes.
- 4) Wash 3 times with PBS, 5 minutes each wash.

8. Nuclear Counterstaining and Mounting

- 1) Hoechst 33342 Staining: Dilute Hoechst 33342(1000×) with PBS at a ratio of 1:1000. Add an appropriate amount of the diluted solution to each sample and incubate at room temperature in the dark for 5-10 minutes.
- 2) Rinse 3 times with PBS, 5 minutes each wash.
- 3) Add an anti-fade mounting medium and seal with a coverslip.
- 4) Observe and capture images immediately under a fluorescence microscope. If immediate observation is not possible, store at 4°C in the dark.

Notes

1. It is recommended to use 4% paraformaldehyde (prepared in PBS). Avoid using ethanol, methanol, or acidic fixatives, as they may significantly reduce labeling efficiency.
2. The permeation time with Proteinase K requires optimization. Excessive permeation may cause sections to detach, while insufficient permeation may result in low labeling efficiency. For first-time experiments, a time-gradient optimization is recommended.
3. All incubation steps must be performed with the samples kept moist. The use of a humidified chamber is recommended, and coverslips or sealing film may be applied during incubations.
4. Azide 488 and Hoechst 33342 fluorescent dyes are light-sensitive and prone to photobleaching. All fluorescence-related steps must be carried out in the dark.

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5. Recombinant TdT Enzyme is temperature-sensitive. Strictly keep it on ice before and after use, and avoid repeated freeze-thaw cycles.
6. Thorough washing after the labeling reaction can effectively reduce background staining.
7. Apoptosis in a very small number of cell types does not involve DNA fragmentation and is therefore not detectable by the TUNEL method. Occasional necrotic cells may also show TUNEL positivity. It is advisable to combine TUNEL results with other apoptosis indicators for confirmatory assessment.
8. This product is for research use only and shall not be used for clinical diagnosis or treatment.

Results Presentation

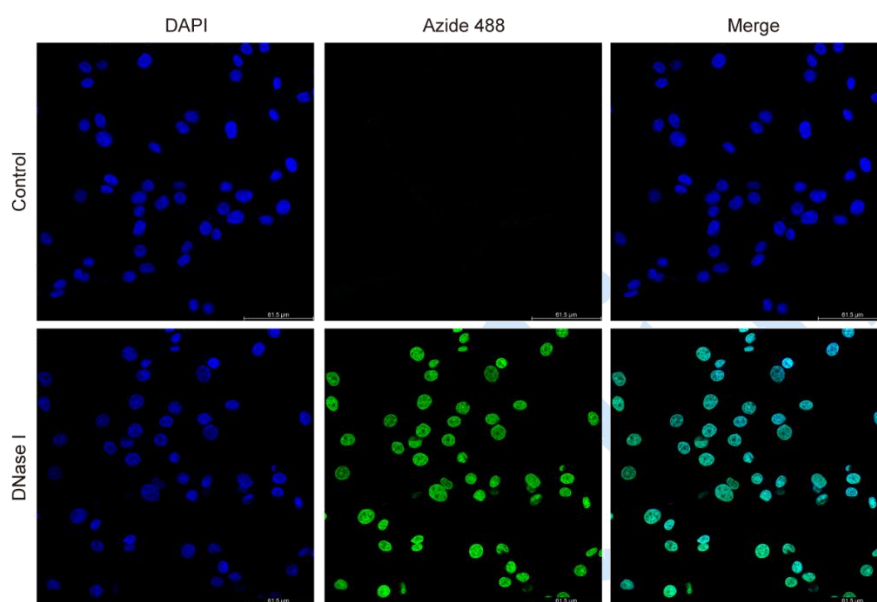


Figure 1. Detection of HeLa cells using the TUNEL Apoptosis Detection Kit. HeLa cells were subjected to TUNEL staining under different conditions. Top panel (Control): Untreated normal HeLa cells. Bottom panel (DNase I): HeLa cells treated with DNase I (D106200) as a positive control for DNA fragmentation. TUNEL-positive cells are shown in green (Azide 488, pseudocolored), and cell nuclei are counterstained with Hoechst 33342 (blue). Merged images (Merge) are shown in the rightmost column.