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TUNEL Chromogenic Apoptosis Detection Kit

Catalog Number: A049

Table 1. Kit Components and Storage

Material	Amount	Concentration	Storage	Stability
TdT reaction buffer (Component A)	**8 mL**	**1X solution**	• **-20 °C,** • **Protect from light**	**The product is stable for 1 year when stored as directed.**
TdT enzyme (Component B)	**100 µL**	**15 U/µL**		
Biotin-11-dUTP (Component C)	**50 µL**	**50X solution**		
HRP-Streptavidin (Component D)	**50 µL**	**100X solution**		
DAB stock solution (Component E)	**150 µL**	**33X solution**		
DAB diluent (Component F)	**5 mL**	**1X solution**		
DNase I (Component G)	**10 µL**	**2 U/µL**		
DNase I buffer (Component H)	**1 mL**	**1X solution**		

Number of assays: 50 assays.

Introduction

Internucleosomal cleavage of DNA is a hallmark of apoptosis. DNA cleavage in apoptotic cells can be detected in situ in fixed cells or tissue sections using the terminal deoxynucleotidyl transferase (TdT) mediated dUTP nick-end labeling (TUNEL) method. TUNEL is highly selective for the detection of apoptotic cells but not necrotic cells or cells with DNA strand breaks resulting from irradiation or drug treatment.

In the TUNEL assay, TdT enzyme catalyzes the addition of labeled dUTP to the 3' ends of cleaved DNA fragments. Hapten-tagged dUTP (e.g. digoxigenin-dUTP or biotin-dUTP) can be detected using secondary reagents (e.g. anti-digoxigenin antibodies or streptavidin) for fluorescence or colorimetric detection. Alternatively, fluorescent dye-conjugated dUTP can be used for direct detection of fragmented DNA by fluorescence microscopy or flow cytometry. The TUNEL Chromogenic Apoptosis Detection Kit contains dUTP conjugated to biotin and HRP-Streptavidin conjugate for high sensitive TUNEL staining.

Materials Required but Not Provided

- Phosphate buffered saline (PBS)
- 4% formaldehyde in PBS
- 0.2% Triton X-100 in PBS
- 3% BSA in PBS
- 2% hydrogen peroxide
- 2X SSC buffer: 300 mM NaCl, 30 mM sodium citrate
- Staining buffer: 0.6 M NaCl, 60 mM sodium citrate, 0.1% Triton X-100, 1% BSA, pH 7.4
- Hematoxylin counterstain

- Mounting medium
- Deparaffinization solvents (Optional)
- Proteinase K (Optional)

Experimental Protocols

Sample Preparation

1. Preparation of cultured cells or fresh-frozen tissue sections

Note: Apoptotic cells can detach from adherent cell cultures and be lost during wash steps. Culture supernatants may be stained using suspension cell protocols to detect detached apoptotic cells.

1.1 Wash cells or sections twice in PBS.

1.2 Fix cells or tissues in 4% formaldehyde in PBS (pH 7.4) for 30 minutes at 4ºC (not required for fixed-frozen sections)

1.3 Wash twice in PBS.

1.4 Permeabilize in PBS containing 0.2% Triton X-100 for 30 minutes at room temperature.

1.5 Wash twice in PBS.

2. Preparation of paraffin tissue sections

2.1 Deparaffinize and rehydrate tissue sections in Coplin jars at room temperature according to Tabel 2, below.

Table 2. Tissue deparaffinization procedure.

Xylenes	Xylenes	100% EtOH	100% EtOH	95% EtOH	85% EtOH	75% EtOH	1X PBS	1X PBS
5 min	5 min	5 min	5 min	5 min	3 min	3 min	5 min	5 min

2.2 Prepare Proteinase K solution at 20 µg/mL in PBS. After use, aliquot any remaining stock solution and store at -20°C.

2.3 Permeabilize sections with 100 µL of 20 µg/mL proteinase K solution for 30 minutes at room temperature. Proteinase K incubation time and temperature may require optimization depending on tissue type. Alternatively, microwave antigen retrieval protocols may be used at this step.

2.4 Rinse in PBS. Wash 2 x 5 minutes in PBS.

Positive Control Preparation

Note: The DNase I generates strand breaks in the DNA to provide a positive TUNEL reaction.

3.1 Wash sample with deionized water.

3.2 Incubate samples with 50 µL DNase I buffer (Component F) for 10 minutes.

3.3 Prepare DNase I solution according to Table 3 and mix well.

Note: Do not vortex the DNase I solution as DNase I denatures with vigorous mixing.

Table 3. DNase I solution.

Reaction Components	**Number of coverslips**		
	1	**2**	**3**
DNase I (Component E)	1 µL	2 µL	3 µL
DNase I buffer (Component F)	49 µL	98 µL	147 µL
Total Volume	50 µL	100 µL	150 µL

3.4 Remove DNase I buffer and add 50 µL of the DNase I solution to each sample and incubate for 30 minutes at room temperature.

3.5 Wash sample once with deionized water.

TUNEL Reaction

4.1 Incubate samples with 2% hydrogen peroxide for 5 min at room temperature to inactivate endogenous peroxidases.

4.2 Rinse in PBS. Wash 2 x 5 minutes in PBS.

4.3 Incubate samples with 100 µL TdT reaction buffer (Component A) for 10 minutes.

4.4 Immediately before use, prepare the TdT reaction cocktail according to Table 4.

Table 4. TdT reaction cocktails.

Reaction Components	**Number of samples**				
	1	**2**	**4**	**5**	**10**
TdT reaction buffer (Component A)	47 µL	94 µL	188 µL	376 µL	470 µL
TdT enzyme (Component B)	2 µL	4 µL	8 µL	16 µL	20 µL
Biotin-11-dUTP (Component C)	1 µL	2 µL	4 µL	8 µL	10 µL
Total Volume	50 µL	100 µL	200 µL	400 µL	500 µL

4.5 Remove TdT reaction buffer and add 50 µL TdT reaction cocktail to each sample, and allow the solution to spread completely over the surface.

a) For adherent cells or tissue sections, cover sample with a Parafilm coverslip to spread buffer evenly over cells or tissue section.

b) For negative control samples, add 50 µL TdT reaction cocktail without TdT Enzyme.

4.6 For cell staining, incubate for 60 minutes at 37ºC, protected from light. Tissue staining may require 2 hours incubation at 37^{o}C.

a) For adherent cells or tissue sections, perform incubation in a humid chamber.

b) For cells in suspension, perform incubation in a microplate on a rocking platform, or resuspend cells in reaction buffer every 15 minutes by gently flicking tubes.

4.7 Stop reaction by incubating samples 2 x 10 minutes in 2X SSC.

4.8 Wash samples 2 x 10 minutes in 3%BSA in PBS.

4.9 Prepare the HRP-Streptavidin staining solution according to Table 5.

Table 5. HRP-Streptavidin staining solution.

Reaction Components	**Number of samples**				
	1	**2**	**4**	**5**	**10**
HRP-Streptavidin (Component D)	1 µL	2 µL	4 µL	5 µL	10 µL
Staining buffer	99 µL	198 µL	396 µL	495 µL	990 µL
Total Volume	100 µL	200 µL	400 µL	500 µL	1000 µL

Staining buffer: 0.6 M NaCl, 60 mM sodium citrate, 0.1% Triton X-100, 1% BSA, pH 7.4.

4.10 Add 100 µL HRP-Streptavidin staining solution to each sample, and incubate for 30 minutes at room temperature, protected from light. Tissue staining may require 1 hours incubation at room temperature.

4.11 Wash samples 2 x 5 minutes in 3%BSA in PBS.

4.12 Prepare DAB staining solution according to Table 6.

Table 6. DAB staining solution.

Reaction Components	Number of samples				
	1	**2**	**4**	**5**	**10**
DAB stock solution (Component E)	3 µL	6 µL	12 µL	15 µL	30 µL
DAB diluent (Component F)	97 µL	194 µL	388 µL	485 µL	970 µL
Total Volume	100 µL	200 µL	400 µL	500 µL	1000 µL

4.13 Add 100 µL DAB staining solution to each sample, and incubate at room temperature. Monitor color development until desired level of staining is achieved (typically 10-60 min). Stop the reaction by rinsing with PBS.

4.14 Counterstain samples with hematoxylin stain if desired. Mount samples in mouting medium for light microscopy.

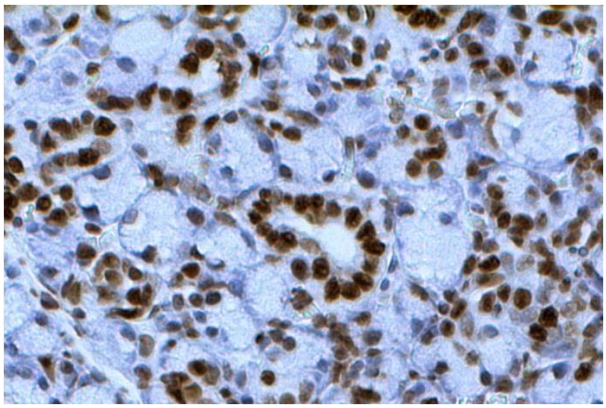

Figure 1. Detection of apoptotic cells in mouse tongue tissue using TUNEL Chromogenic Apoptosis Detection Kit.