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TUNEL Apoptosis Assay Kit

Product name: TUNEL Apoptosis Assay Kit
Catalog No.: TAAP01D

Introduction:

The BIOTnA TUNEL Apoptosis Assay designed to detect apoptosis in fixed cells, embedded tissue, and frozen tissue using light microscopy. DNA fragmentation represents a characteristic hallmark of apoptosis. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) is an established method for detecting DNA fragments. In this modified TUNEL assay kit, Digoxigenin-nucleotide is labeled at the 3′-OH ends of DNA by using the natural or recombinant terminal deoxynucleotidyl transferase (TdT or rTdT). The mouse anti- DIG and HRP labeled polymer bind to these Digoxigenin nucleotides, which are detected by following the peroxidase substrate, 3,3'-diaminobenzidine (DAB). The nuclei of apoptotic cells should be observed dark brown to bluish black under light microscope.

Form:

Catalog No.	Size
TAAP01D-20	20 reactions
TAAP01D-50	50 reactions
TAAP01D-100	100 reactions

Kit Contents:

Kit Contents	Format	Recommend time	Storage
Proteinase K	Ready to Use	10-30 minutes.	4℃
Permeabilization buffer	Ready to Use	10-30 minutes.	4℃
TdT Reaction Buffer	Ready to Use	10 minutes.	4℃
TdT Enzyme Reagent	Concentrate	Diluted in TdT Label Reagent	-20℃
TdT Label Reagent	Ready to Use	60 minutes.	-20℃
Background reducing buffer	Ready to Use	30 minutes.	4℃
anti- DIG	Ready to Use	60 minutes.	4℃
HRP labeled polymer	Ready to Use	30 minutes.	4℃
DAB Chromogen (33x)	Concentrate	Diluted in DAB Buffer	4℃
DAB Buffer	Ready to Use	1-10 minutes.	4℃

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Protocol for FFPE tissue sections

Staining Protocol Recommendations:

1. Deparaffinize and rehydrate formalin-fixed paraffin-embedded tissue section.
2. Add enough drops of 3% Hydrogen Peroxide Block to cover the sections. Incubate for 10 minutes. Wash in PBS buffer for 3x2 min.
3. Pretreatment: add 50ul proteinase K for 10-30 minutes to digest sample.
4. Wash in PBS buffer for 2 min.
5. Apply enough Permeabilization buffer to cover the tissue and incubated for 30 minutes at room temperature.
6. Wash in PBS buffer for 3x2 min.
7. Pre-incubation: incubate sections in TdT Reaction Buffer for 10 minutes.
8. Throw the TdT Reaction Buffer away and Do Not wash.
9. TdT Reaction: incubate sections in TdT Reaction Mixture for 1 hours at 37 °C in humidified chamber. (Working buffer: TdT Enzyme Reagent 5ul mixed in TdT Label Reagent 45ul).
10. Wash in PBS buffer for 3x2 min.
11. Apply 50ul Background reducing buffer and incubate for 30 minutes at room temperature to block non-specific background staining. (Mouse tissues and some tissues need).
12. Wash in PBS buffer for 3x2 min.
13. Apply 50ul anti- DIG and incubate for 1 hours at room temperature in humidified chamber. Wash 3 times in PBS buffer.
14. Apply 50ul HRP labeled polymer and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
15. DAB Chromogen / DAB Buffer: add 50ul DAB working buffer and incubate sections with DAB for 5-20 minutes. (DAB working solution: 1ml DAB Buffer add 30ul DAB Chromogen)
16. Counterstain with hematoxylin for 10 seconds.
17. Rinse in running tap water for 5 minutes.
18. Dehydrate through 95% ethanol for 2min, 100% ethanol for 2x3min.
19. Clear in xylene for 2x5min.
20. Coverslip with xylene based mounting medium.

Protocol for fixed cells

Sample material: 1. Adherent cells cultured on chamber slides
2. Cells in suspension
3. Cytospin and cell smear preparations

Sample Preparation and Fixation

1. Adherent cells cultured on chamber slides

For optimal outcomes, cells should be grown on a surface that allows for both fixation and direct labeling, such as sterile chamber slides. Cells can be cultured directly on sterile coverslips that are placed into a 6- or 12-well tissue culture plate.

Method

1. Remove media from cells and rinse once with 1X PBS at room temperature.
2. Fix cells for 10 minutes at room temperature in 3.7% buffered formaldehyde.
3. Wash cells one time in 1X PBS. Samples can be stored at this point, using one of the following methods:
   a) Dehydrate the cells by passing through an increasing alcohol series of 70%, 95%, and 100% ethanol for 5 minutes each followed by air drying for 10 minutes. Store at 4 °C with desiccant.
   b) Fixed cells can be stored for up to 1 week in 0.5% buffered formaldehyde at 4 °C. The samples must be covered to prevent contamination and evaporation. If experimental design dictates a time course extending over several days, storage in 0.5% buffered formaldehyde is recommended.

Note: labeling directly after fixation is optimal as the labeling of some samples is less efficient after storage. If possible, a pilot study should be performed to ensure that stored fixed samples can be labeled.

4. Proceed to Labeling Procedure.

2. Cells in suspension

Cells grown in suspension or prepared from dissociated tissues can be fixed in solution, and then spotted onto pretreated glass microscope slides for processing. This method is quick and easy and requires no special equipment. Cells immobilized onto glass slides can be stored for several months.

Method

1. Harvest cell suspension by centrifugation at 500 x g for 5 minutes at room temperature.
2. Discard media and resuspend at 1 x 106 cells per ml in 3.7% buffered formaldehyde. Let stand for 10 minutes at room temperature.
3. Centrifuge at 500 x g for 5 minutes at room temperature and discard fixative.
4. Resuspend at 1 x 10^7 cells per ml in 80% ethanol. Cells can be stored in 80% ethanol at 4 °C for several weeks. However, signal intensity in positive cells will reduce with time due to loss of small DNA fragments.
5. Spot 1 x 10^5 cells onto clean glass microscope slide. Dry for 20 minutes on slide warmer at 45 °C.

Note: Glass slides pretreated for electrostatic adherence are recommended (e.g. Microscope Slides). Other slide treatments (e.g. collagen, gelatin, poly-L-lysine) can cause increased background staining.

6. Immerse slide in 70% ethanol for 10 minutes, then air dry overnight at room temperature or dry at 45 °C for 2 hours. Samples may be stored at this point. Store samples at 4 °C in airtight containers with desiccant for up to several months.

7. Proceed to Labeling Procedure.

3. Cytospin and cell smear preparations

Method

1. The cells prepared by cytospin and cell smear will allow to air dry.
2. Fix air dried cell sampless for 10 minutes at room temperature in 3.7% buffered formaldehyde.
3. Wash cells one time in 1X PBS. Samples can be stored at this point, using one of the following methods:
   a) Dehydrate the cells by passing through an increasing alcohol series of 70%, 95%, and 100% ethanol for 5 minutes each followed by air drying for 10 minutes. Store at 4 °C with desiccant.
4. Proceed to Labeling Procedure.

Rehydrate

After storage, rehydrate through a decreasing alcohol series and wash in 1X PBS prior to the labeling reaction.

7. Rehydrate by immersing for 5 minutes each in 100%, 95%, then 70% ethanol.
8. Immerse in 1X PBS and proceed to Labeling Procedure.

Labeling Procedure Recommendations:

1. Rehydrated the fixed cell.
2. Add enough drops of 3% Hydrogen Peroxide Block to cover the cell slides. Incubate for 5 minutes (Do not leave longer than 5 minutes since hydrogen peroxide can damage DNA). Wash in PBS buffer for 3x2 min.
3. Permeabilisation: incubate with 0.2% Triton X-100 in PBS-Tween for 30 minutes
4. Pretreatment: add 50ul proteinase K to digest sample.
5. Wash in PBS buffer for 3x2 min.
6. Apply 50ul Background reducing buffer and incubate for 30 minutes at room temperature to block non-specific background staining. Wash in PBS buffer for 3x2 min.
7. Pre-incubation: incubate cell slides in TdT Reaction Buffer for 10 minutes.
8. TdT Reaction: incubate cell slides in TdT Reaction Mixture for 1-2 hours at 37°C in humidified chamber. (Working buffer: TdT Enzyme Reagent 5ul add TdT Label Reagent 45ul).
9. Wash in PBS buffer for 3x2 min.
10. Apply 50ul mouse anti- DIG and incubate for 1 hours at 37°C in humidified chamber. Wash 3 times in PBS buffer.
11. Apply 50ul HRP labeled polymer and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
12. DAB Chromogen / DAB Buffer: add 50ul DAB working buffer and incubate sections with DAB for 5-20 minutes. (DAB working solution: 1ml DAB Buffer add

30ul DAB Chromogen)

13. Counterstain with hematoxylin for 30 seconds.
14. Rinse in tap water for 2 minutes for 3 times.
15. Dehydrate through 95% ethanol for 2min, 100% ethanol for 2 min. for 3 times.
16. Clear in xylene for 2x5min.
17. Coverslip with xylene based mounting medium.

Storage and Stability:

Please read the kit contents and follow the storage condition. The user must validate any other storage conditions. When properly stored, the reagent is stable until the date indicated on the label. Do not use the reagent beyond the expiration date. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the reagent is suspected, contact Technical : info@biotna.net