

TUNEL Apoptosis Assay Kit

Product name: TUNEL Apoptosis Assay Kit

Catalog No.: TAAP01D

Introduction:

The kit can detect fragmented DNA in the nucleus during apoptosis. In this modified TUNEL assay kit, Digoxigenin-nucleotide is labeled at the DNA 3'-OH ends using the natural or recombinant terminal deoxynucleotidyl transferase (TdT or rTdT). Then, mouse anti-DIG and HRP labeled polymer bind to these Digoxigenin nucleotides, which are detected using the peroxidase substrate, 3,3'-diaminobenzidine (DAB), a stable chromogen. Using this procedure, apoptotic nuclei are stained dark brown.

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°C
Permeabilization buffer	Ready to Use	10-30 minutes.	4°C
TdT Reaction Buffer	Ready to Use	10 minutes.	4°C
TdT Enzyme Reagent	Concentrate	Diluted in TdT Label Reagent	-20°C
TdT Label Reagent	Ready to Use	60 minutes.	-20°C
Background reducing buffer	Ready to Use	30 minutes.	4°C
anti-DIG	Ready to Use	60 minutes.	4°C
HRP labeled polymer	Ready to Use	30 minutes.	4°C
DAB Chromogen (20X)	Concentrate	Diluted in DAB Buffer	4°C
DAB Buffer	Ready to Use	1-10 minutes.	4°C

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.
3. Wash in PBS buffer for 2 min(3 times).
4. Pretreatment: add 50ul proteinase K for 10 minutes to digest sample.
5. Wash in PBS buffer for 2 min.
6. Apply enough Permeabilization buffer to cover the tissue and incubated for 30 minutes at room temperature.
7. Wash in PBS buffer for 2 min(3 times).
8. Apply 50ul Background reducing buffer and incubate for 30 minutes at room temperature to block non-specific background staining. (It is necessary for mice tissue and others with non-specific background.)
9. Wash in PBS buffer for 2 min(3 times).
10. Pre-incubation: incubate sections in TdT Reaction Buffer for 10 minutes.
11. Throw the TdT Reaction Buffer away from the tissue and Do Not wash.
12. 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).
13. Wash in PBS buffer for 2 min (3 times).
14. Apply 50ul anti- DIG and incubate for 1 hours at 37 °C in humidified chamber. Wash 3 times in PBS buffer.
15. Apply 50ul HRP labeled polymer and incubate for 30 minutes at room temperature. Wash 3 times in PBS buffer.
16. DAB Chromogen / DAB Buffer: add 50ul DAB working buffer and incubate sections with DAB for 5-20 minutes. (DAB working solution: 950ul DAB Buffer add 50ul DAB Chromogen)
17. Counterstain with hematoxylin for 10 seconds.
18. Rinse in running tap water for 5 minutes.
19. Dehydrate through 99% ethanol for 2min.
20. Air dry and 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