

# Detection of TUNEL in Formalin-Fixed, Paraffin-Embedded Mouse and Rat Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: ApopTag® Plus Peroxidase In Situ Apoptosis Kit

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Catalog # S7101

**Note:** This kit contains all reagents necessary to perform the stain. However, this protocol uses a chromogen different from the one provided in the kit.

## Staining Procedure

Positive Control Tissue: Ovary – apoptotic bodies  
Stain Localization: Varies

**Note: Specified reagent volumes ensure 40 slides per kit when using 5 cm<sup>2</sup> sections.**

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.

3. Rinse slides in 2 changes of 1X wash buffer for 5 minutes each.

4. Heat-Induced Epitope Retrieval Using The Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer  
(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* \_\_\_\_\_  
Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* \_\_\_\_\_

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

5. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

ApopTag® TUNEL Kit

Exp Date \_\_\_\_\_ New Kit: yes / no

6. Apply 75 µl of Equilibrium Buffer (Ready-to-use) to the slides. Incubate for 10 minutes at room temperature.

**DO NOT RINSE SLIDES. GENTLY TAP OFF EXCESS REAGENT AND CAREFULLY BLOT AROUND THE TISSUE SECTIONS.**

7. Apply 55 µl of TdT enzyme solution to the slides. Incubate for 15 minutes at 37°C.

(Solution made by mixing 33 µl of TdT with 77 µl of Reaction Buffer – a 1:3 dilution. Adjust the volumes proportionately based on the number of slides being stained.)

8. Rinse the slides in Stop/wash buffer solution for 10 minutes, agitating the slides the first 10 seconds to stop the reaction. (Solution made by adding 1 ml of Stop/wash buffer per 34 ml of distilled water.)

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.

10. Apply 65  $\mu$ l of the Anti-Digoxigenin-Peroxidase Reagent (Ready-to-use) to the slides. Incubate for 30 minutes at room temperature.
11. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
12. Apply the DAB chromogen. Incubate in the dark for 6 minutes at room temperature.  
(Add 1 drop of DAB per ml of substrate)  
Lot #\_\_\_\_\_ Exp. Date\_\_\_\_\_ New Kit: yes / no
13. Rinse the slides in tap water for 3 minutes.
14. Counterstain with hematoxylin for 20 seconds.
15. Rinse the slides in tap water until water is clear.
16. Gently agitate slides in 1X wash buffer until the tissues turn blue.
17. Dehydrate through the following solutions:

<b>Solutions</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

18. Coverslip

*Updated 05/21/13*