

Pathology Core Protocol



Protocol Section: Staining Procedures Manual
Protocol Subject: TUNEL

Policy No: P-CMHD2-Tun-00
Effective Date:
Date Reviewed: 6 January 2003
Date Revised:

TUNEL Labeling on paraffin section

Material:

10x One-Phor-All buffer (Pharmacia, come with TdT enzyme)
 TdT enzyme, FPLCpure™ (Pharmacia, 27-0730-02)
 Biotin-16—d UTP (Roche, 1 093 070): dilute 1: 6 with dd H₂O
 100 mM d ATP (Pharmacia, 27-0730-02)
 0.1% Triton X- 100 in dd water
 0.1% Triton X- 100 in PBS
 VectaStain ABC complex (mouse ABC kit)

Protocol for paraffin section

1. De-wax and rehydrate:
 - Xylene 2 x 5 minutes
 - 100% ethanol 2 x 5 minutes
 - 90% ethanol 1x 3 minutes
 - 80% ethanol 1x 3 minutes
 - 70% ethanol 1x 3 minutes
 - dd water 1x 3 minutes
 - PBS 1x 3 minutes
2. Enzyme digest: 10 µg/ml proteinase K in PBS
3. Wash: dd water 5 minutes, PBS 2x5 minutes.
4. Quench endogenous peroxidase: 3% hydrogen peroxide in methanol 30 minutes at RT
5. Wash in PBS 3x 5 minutes
6. Pre-incubate section with 1x One –Phor-All buffer (10 x stock diluted to 1 x with 0.1% Triton X-100 in dd water) for 10 minutes.
7. Prepare TdT solution 100 µl for 2-3 slides
 - 10 µl 10x One-Phor-All buffer
 - 6 µl dilute biotin-16-d UTP
 - 1 µl 100 µ M d ATP
 - 1 µl TdT enzyme
 - 82 µl 0.1% Triton X- 100 in dd water
8. Incubate in moist chamber for 2 hours at 37 cent grade.
9. Prepare ABC complex solution before use 30 minutes. A and B both 1: 50

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10. Wash in PBS 3x 5 minutes
 11. Add ABC complex solution and incubate at 37 cent grade for 90 minutes
 12. Wash in 0.1% Triton X-100 in PBS 2x 5 minutes , and PBS 2x 5 minutes
 13. DAB color develop 15 minutes
 14. Counterstain with hematoxylin for 20 seconds. Acid alcohol 3 dips
 15. Dehydrating and clearing.
 16. Mounting

Issued by Lab Manager: _____ **Date:** _____

Approved by Facility Management: _____ **Date:** _____