

TUNEL PROTOCOL

Quick protocol based on **TACS-XL® DAB *In Situ* Apoptosis Detection Kit** (cat. No. 4828-30-DK). For more detailed instructions, see kit manual.

TACS-XL® DAB kits & protocol also available for Blue Label. We have a range of tissue specific apoptosis detection kits based on the TUNEL assay (enquire for details or download our TUNEL Brochure).

- 1. Immerse hydrated, fixed and immobilized sample in 1X PBS for 10 minutes.
- 2. Cover sample with 50 µl of Proteinase K Solution (*) for 15-30 minutes.

Proteinase K Solution per sample:

- 50 µl Apoptosis Grade Water
- 1 µl Proteinase K

OR Cover sample with 50 µl of Cytonin™ for 30 minutes.

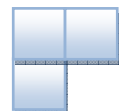
- 3. Wash two times in deionized water, 2 minutes each.
- 4. Immerse sample in Quenching Solution for 5 minutes.
 - 45 ml Methanol
 - 5 ml 30% hydrogen peroxide
- 5. Wash samples in 1X PBS for 1 minute.
- 6. Immerse sample in 1X TdT Labeling Buffer for 5 minutes.
 - 45 ml Deionized water
 - 5 ml 10X TdT Labeling Buffer
- 7. Cover sample with 50 µl of Labeling Reaction Mix (*). Incubate for 30 minutes at 37°C in a humidity chamber.

Labeling reaction mix per sample:

- 1 µl B-dNTP Mix
- 1 µl TdT Enzyme
- 50 µl 1X TdT Labeling Buffer

A positive control can be generated at this step using TACS-Nuclease (refer to Section VII. Controls, on page 16 of detailed protocol).

- 8. Immerse sample in 1X TdT Stop Buffer for 5 minutes.
 - 45 ml Deionized water
 - 5 ml 10X TdT Stop Buffer
- 9. Wash two times in 1X PBS, 2 minutes each.



- 10. Cover sample with 50 μ l of Antibody Solution (*). Incubate for 30 minutes at 37°C.

Antibody Solution per sample:

- 50 μ l Strep-Diluent
- 1 μ l Anti-BrdU

- 11. Wash 3 times in 1X PBST, 2 minutes each.

- 12. Cover sample with 50 μ l of Strep-HRP Solution (*). Incubate for 10 minutes.

Strep-HRP Solution per sample:

- 50 μ l 1X PBS
- 1 μ l Strep-HRP

- 13. Wash 3 times in 1X PBS, 2 minutes each.

- 14. Immerse in DAB solution for 2 to 7 minutes.

- 50 ml 1X PBS
- 250 μ l DAB
- 50 μ l 30% hydrogen peroxide

- 15. Wash in deionized water 2 times, 2 minutes each.

- 16. Immerse in 1% Methyl Green for 30 seconds up to 5 minutes.

- 17. Dip slides ten times each in 2 changes of deionized water, 95%, then 100% ethanol.

OR Dip slides ten times in 2 changes 1-butanol.

- 16. Dip 10 times each in 2 changes of xylenes.

- 17. Mount glass coverslip using mounting medium.

(*) - Hydrophobic coverslips may be used for small reagent volumes.

