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TUNEL Staining – In Situ Cell Death Detection Kit[1] Protocol

1. Permeabilise floating sections in assay buffer[2] with 0.3%-1.0% Triton X-100 for 1 hour to 18 hours (overnight) at 4° to 24°C.

2. Mount floating sections onto gel-subbed slides. Allow to air dry for at least an hour at 37°C.

3. Rinse slides twice with PBS.

4. Dry area around sections.

Preparation of TUNEL reaction mixture[3]

   a. Remove 100ul Label Solution from bottle 2 for negative controls.
   b. Add total volume of bottle 1 (50ul) to the remaining 450ul Label Solution in bottle 2 to obtain 500ul TUNEL reaction mixture.
   c. Mix well to equilibrate components.

5. Add TUNEL reaction mixture on sections. Add 100ul Label Solution on negative control sections. Incubate sections in a humidified chamber for 60 min at 37°C.

6. Rinse slides 3 times with PBS.

7. ***Sections can be analyzed under a fluorescence microscope with a FITC filter[4].***

The following are steps to make labeling permanent:

8. Dry area around sections.

9. Add Converter-AP[5] on sections. Incubate sections in a humidified chamber for 30 min at 37°C.

10. Rinse slides 3 times with PBS.

Preparation of Vector Blue Alkaline Phosphatase Substrate Solution Kit III[6]

   a. Add 2 drops of Reagent 1 to 5ml of 100mM Tris-HCl, pH 8.2 buffer. Mix well.
   b. Add 2 drops of Reagent 2. Mix well.
   c. Add 2 drops of Reagent 3. Mix well.

11. Add substrate solution on sections. Incubate sections with substrate solution at room temperature until suitable staining develops. Development times should be determined by the investigator but generally 20-30 min provides good staining intensity. *Improved staining may be obtained by developing the substrate in the dark*.

12. Wash sections in assay buffer for 5 min. Rinse slides in tap water.

13. Vector Blue is partially soluble in xylene; avoid xylene-based clearing agents and mounting media. Dehydrate sections and mount in a non-xylene-based mounting media.

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[1] Roche: Cat. No. 1 684 809 - *http://biochem.boehringer-mannheim.com/proddata/intnl/3_5_3_21_2_27.cfm*
[2] Tris buffer or PBS
[3] The TUNEL reaction mixture should be prepared immediately before use and should not be stored. Keep on ice until use.
[4] Use an excitation wavelength in the range of 450-500nm and detection in the range of 515-565nm (green).
[5] The Converter-AP is included with the In Situ Cell Death Detection Kit. However, it is stored elsewhere at 4°C.
[6] Vector: Cat. No. SK-5300 - *http://www.vectorlabs.com/Substrates/SK5300.html*

*Protocol adapted from instructions found in the **In Situ Cell Death Detection Kit** and the **Vector Blue Alkaline Phosphatase Substrate Kit III**. Please refer to these for complete instructions.*

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