TUNEL assay using ApopTag Kit from Intergen Company (Cat #S7110)

1. **Deparaffinize tissue sections** (in a coplin jar)
    a. Wash slides with xylene, 5 min twice.
    b. Rehydrate in a decreasing ethanol series: 100% x2, 95% x2, 70%, 2 min each.
    c. Rinse in PBS, 3 min x 3
2. **Pretreat tissues**
    a. Incubate slides in Proteinas K (20 μg/ml final concentration) for 15 min at RT. To prepare 1 ml of ProK solution (good for two slides), add 4 μl 25 mg/ml ProK to 1 ml of PBS and mix. Optimal ProK treatments need to be tested for frozen sections.
    b. Rinse well in PBS 2 x 2 min.

3. **Apply Equilibration Buffer** (blue cap)
    a. Gently tap off excess liquid and carefully blot or aspirate around the section.
    b. Immediately apply 75 μl Equilibration Buffer directly on the specimen, and coverslip.
    c. Incubate for at least 10 sec at RT.

4. **Apply TdT enzyme** (for 2 slides, 33 μl TdT Enzyme + 77 μl Reaction Buffer, mix and keep on ice, good for 6hr)
    a. Gently tap off excess liquid and carefully blot or aspirate around the section.
    b. Immediately pipette onto the section 55 μl/5 cm^2 of working strength TdT enzyme and coverslip.
    c. Incubate in a humidified chamber at 37°C for 1 hour.

5. **Apply Strop/Wash Buffer** (1ml Stop/Wash Buffer + 49 ml H_2O)
    a. Put the slides in working strength Stop/Wash Buffer in a coplin jar), agitate for 15 sec, and incubate for 10 min at RT.
    b. Remove an aliquot of Anti-Digoxigenin Conjugate from the stock vial sufficient to process the desired number of slides. (Warm the aliquot to RT while avoiding exposure to light.
6. **Apply Anti-Digoxigenin Conjugate** (for 2 slides, 68 µl blocking Solution + 62 µl Anti-Digoxigenin Conjugate, votex and keep on ice in dark)
    a. Wash the slides in PBS 3 x 1 min.
    b. Gently tap off excess liquid and carefully blot or aspirate around the section.
    c. Apply warmed (RT) working strength Anti-Digoxigenin Conjugate (rhodamin) to the slide; use about 65 µl/5 cm^2 of specimen surface area and coverslip.
    d. Incubate in a humidified chamber **in dark** for 30 min. at RT.

7. **Counter staining**
    a. Wash slides in PBS, 2 min x 4.
    b. Add 0.5 ml of Hoechst solution to each slides and incubate for 10 min in dark. To prepare Hoechst solution, mix 1 μl of stock solution (10 mg/ml, Hoechst 33342, Molecular Probes, Cat. # H-3570) with TBS (PBS containing 01.% Triton-100).
    c. Wash with PBS 5 min x 3.
    d. Mount slides with Gel mount or ProLong Antifade Kit (Molecular Probes, Cat. # P-7481).
    e. Seal slide with nail polisher next morning.