

BrdU Immunofluorescent staining

Day one

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%, 50% 2 min each
 - c. Rinse in PBS, 3 min x 3

2. Pretreat tissues
 - a. Incubate slides in 0.002% pepsin (Sigma), 0.01 N HCl in PBS (70 μ l of 2% pepsin in pre-warmed 70 ml of 0.01N HCl in PBS) for 5min at 37°C.
 - b. Denature DNA in 2 N HCl (12 ml concentrated HCl into 68 ml H₂O), for 10 min at RT.
 - c. Neutralize in 0.1 M sodium borate, pH 8.5, 10 min.
 - d. Wash with PBS for 10 min.

3. Blocking and incubation with primary antibody
 - a. Incubate slides with 1% of horse serum at RT for 30 min.
 - b. Incubate with mouse anti-BrdU IgG1 (Becton-Dickinson #347580) as the primary Ab at 1:20 in the blocking solution, in a humidified chamber, 4°C, O/N (5 hr minimum). As a control for Ab specificity, leave blocking solution alone on one slide, without antibody.

Day two

4. Incubation with secondary Ab **in dark**.
 - a. Allow slides to warm up at least 45 min at RT.
 - b. Remove the primary Ab, and rinse in PBS 3 min x 5.
 - c. Incubate all slides with secondary Ab for 1 hr at RT in a humidified chamber **in dark**. One drop normal horse serum in 10 ml PBS, vortex, add 1:250 ratio of fluorescent Ab, vortex.
[1.5ml of hs mix + 6 μ l of fluorescent Ab = 3 slides] 0.5 ml of Ab per slide

5. Counter staining **in dark**
 - a. Wash slides in PBS, 2 min x 4.
 - b. Incubate slides in 0.1% Triton in PBS, (70 μ l of Triton into 70 ml of PBS), and add 7 μ l of stock HOECHST solution (conc 10 mg/ml) (Hoechst 33342, Molecular Probes, Cat. # H-3570) **in dark for 10 min**
 - 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.

Note: Do not let the sections dry between steps!