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HCL-free detection of BrdU by immunocytochemistry:

1. BrdU-label cells as fix 4% PF as usual
2. Wash PBS
3. enzyme (DNAase I @ 1000 Kunitz/ml in PBS with 4.2mM $MgCl_2$ – $MgCl_2$ is in anhydrous powder form in chemical cabinet, make a 100mg/ml stock solution in DI water for diluting in PBS) x 1hr, 37°C (use the bacterial incubator)
4. wash cold PBS
5. Block: overnight 4° x in PBS/0.2% triton/10% Goat Serum
6. 1°: mouse anti-BrdU (1:50) x overnight 4°, in PBS/0.2% triton/2% GS
7. wash 3 x 10' in PBS/0.2% T
8. 2°: goat anti-mouse (1:200-1:500) in PBS/0.2% triton/2% GS, x 1hr
9. wash 3 x 10' in PBS
10. Continue with other stains

Deoxyribonuclease I: Sigma (D4263), vial of 2,000 Kunitz .

Note: a *Kunitz* is defined as the amount of enzyme required to produce a $Delta_{260}$ of 0.001 per min per ml at pH 5.0 at 25°C, using DNA type I or III as a substrate, with [Mg++] = 4.2 mM.

Storage and Working Dilution: Remember when making your working dilution that the concentration of DNAse is not the important factor, the amount of DNAse is what you want to control from sample to sample. Zhenjie Lu used about 10 kunitz per sample for epithelial sheets and got excellent results. Higher amounts may be necessary for whole mount samples. Find the amount that works best for your specific application and be sure to use the same volume every time (I use about 100ul in a 1.5ml centrifuge tube). Remember that when storing the DNAse after diluting it in PBS, it begins to lose activity after one week even at -20°, so make dilutions and aliquots accordingly. Also, do not add Mg to the dilution until you are ready to use it as adding it before storage will only cause additional loss in activity.