

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 MgCl₂ – MgCl₂ 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 Delta₂₆₀ 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 andT 0 0 1 72.0(lease)5(I)4 409.75.aufa[(S)-3(tora)5(g)10(e)4(04st)-3(6.85 in[(C)-2(, usi)-2(1250n)9(C