

BrdU staining with HCL (single staining):

1. Incubate cells in BrdU (10 μ M BrdU 30 min-2 hrs, longer if needed).
2. Wash in PBS 3X
3. Fix in 2-4% PFA (5 min max)
4. Wash in PBS
5. Denature in HCl (2M). Incubate at 37°C for 30 min (or 1 hr @ RT).
7. Rinse HCl off. Neutralize with 0.1 M Borate Buffer (PH=8.5) X3 (1mL per well). PBS 1X works as well
7. Neutralize with 0.1 M Borate Buffer (PH=8.5) X3 (1mL per well). PBS 1X works as well, do at least 3X 5 min washes!
8. Wash with PBS X3.
9. Add 200ml PBS/T20/2%Serum. Incubate at 37°C for 30 minutes. (or Blocking buffer).
10. Aspirate off the mixture. Add anti-BrdU antibody. (1:50-1:200).
11. Incubate at 1-2 hrs @ RT.
11. Wash with PBS/T20 X3.
12. Add secondary antibody, 45' @RT.
13. Wash with PBS/T20 X3.
14. Add DAPI, wash/ mount