

**LP110 – BrdU IMMUNOHISTOCHEMISTRY STAINING
ON FREE-FLOATING FROZEN SECTIONS**

We describe below general recommendations for antibody staining of BrdU. Each antibody reacts differently on different tissues, therefore different protocols and conditions have to be tested to obtain the optimal results for end-user application.

Protocol

1. Wash with PBS three times 3 min each
2. Treat with Hydrogen Peroxide (3% in PBS) for 15 min
3. Wash with PBS three times 3 min each
4. Permeabilize with 0.2% Triton X-100 for 30 min
5. Wash with PBS three times 3 min each
6. Denature DNA with 2N HCl for 10 min
7. Wash with PBS three times 3 min each
8. Neutralize HCl with 0.1M Sodium Tetraborate (pH 8.5) for 10 min
9. Wash with PBS three times 3 min each
10. Preincubate with 5% Normal Horse Serum in PBS for 60 min
11. Incubate with primary antibody, overnight, room temperature
Rabbit anti BrdU (Abbotec, 250563)
Dilution: 1/200
Diluent: 5% Normal Horse Serum in PBS
12. Wash with PBS (0.01 M, pH 7.4) five times 5 min each
13. Preincubate with 5% Normal Horse Serum in PBS for 60 min
14. Incubate with secondary antibody for 90 min, room temperature
Donkey anti Rabbit
(Jackson, biotinylated, #711-066-152)
Dilution: 1/2000
Diluent: 5% Normal Horse Serum in PBS
15. Wash with PBS five times 5 min each.
16. Incubate with Streptavidin HRP for 90 min, room temperature
(Jackson, #016-030-084)
Dilution: 1/5000
Diluent: 1% Normal Horse Serum in PBS
17. Wash with PBS five times 5 min each
18. Wash with TB (0.05 M, pH 7.6) for 5 min
19. Develop signal with DAB + Nickel Chloride for 1 min
20. Wash with TB (0.05 M, pH 7.6) for 5 min
21. Wash with PBS two times 5 min each
22. Mount sections from Gelatin (0.5%), let them air-dry
23. Dehydrate with Ethanol (70-90-96-100-100-100%)
24. Clear with Xylene twice
25. Coverslip slides with DPX



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Protocol

1. Wash with PBS three times 3 min each
2. Permeabilize with 0.2% Triton X-100 for 30 min
3. Wash with PBS three times 3 min each
4. Denature DNA with 2N HCl for 10 min
5. Wash with PBS three times 3 min each
6. Neutralize HCl with 0.1M Borate buffer (pH 8.5) for 10 min
7. Wash with PBS three times 3 min each
8. Preincubate with 5% Normal Horse Serum in PBS for 60 min
9. Incubate with primary antibody, overnight, room temperature
Rabbit anti BrdU (Abbotec, 250563)
Dilution: 1/100
Diluent: 5% Normal Horse Serum in PBS
10. Wash with PBS five times 5 min each
11. Preincubate with 5% Normal Horse Serum in PBS for 60 min
12. Incubate with secondary antibody for 90 min, room temperature
Donkey anti Rabbit
(Jackson, Cy2 conjugated, #711-226-152)
Dilution: 1/500
Diluent: 5% Normal Horse Serum in PBS
13. Wash with PBS five times 5 min each.
14. Counterstain sections with Hoechst 33342 (Invitrogen, #H-3570) for 5 min
Dilution: 1/1000
Diluent: PBS
15. Wash with PBS three times 3 min each
16. Mount sections from PBS, let them air-dry
17. Coverslip slides with Fluoromount-G (Sigma, #F4680)
18. Drain the excess Fluoromount-G
19. Store slides at 4°C

Solution Preparation

2N HCl:

add 167 ml cc. HCl to 833 ml distilled water. Mix well.

0.1M Borate Buffer, pH 8.5:

Sodium borate (Sigma, S9640, MW 381.4) 38 g
Mix to dissolve in 800 ml distilled water
adjust pH to 8.5 with HCl
make 1000 ml final volume with distilled water
Prepare fresh before use.