BrdU Labeling of HeLa Cells Followed by Immunostaining

Protocol

Immunofluorescence

For use with immunocytochemistry tested Rabbit Anti-BrdU Antibody. This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that a certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques - these are guidelines only and may need to be adjusted for particular applications. Note that this protocol has been developed for the BrdU labeling and immunostaining of HeLa cells. Therefore, the protocol will need to be optimized for use in other cell types.

Reagents

- 5′-bromo-2′-deoxyuridine (BrdU)
- Fetal bovine serum (FBS)
- Formaldehyde
- Hydrochloric acid (HCl)
- Immunofluorescence buffer (IF buffer) (prepare by adding 0.75 g glycine per 100 ml phosphate buffered saline)
- PBS
- Rabbit Anti-BrdU Antibody (catalog #AHP2405)
- Triton X-100

(Optional) PureBlu™ DAPI Nuclear Staining Dye and/or anti-actin/GAPDH/tubulin antibodies to stain cytoplasmic proteins

Method

BrdU labeling (adapted from O'Keefe RT et al. (1992))

1. Seed HeLa cells at a density of 1.25 x 10^5 cells/ml in culture plates and allow cells to grow for 24-48 hr before staining.
2. As with every experiment, include appropriate positive and negative controls, such as solvent-only controls. For more information about experimental design tips, refer to our application resources.
3. Remove culture medium.
4. Add cell culture medium containing 10% FBS and 10 µM BrdU to cells.
5. Incubate for 1-3 hr at 37°C.
6. Wash the cells 3 times (3x) with 1x PBS.
7. Add 2% formaldehyde in PBS (pH 7.4).
8. Incubate for 15 min at RT.
9. Wash cells 3x with 1x PBS.

BrdU immunostaining

10. Add 0.2% Triton X-100 in PBS.
11. Incubate for 5 min at RT.
12. Wash 3x with 1x PBS.
13. Add 2 M HCl to denature the DNA*.
14. Leave for 30 min at RT.
15. Wash 3x with 1x PBS.
16. Wash cells 1x with IF buffer.
17. Incubate cells in IF buffer for 30 min at RT.
18. Remove excess liquid.
19. Incubate the cells with 10% FBS in PBS for 30 min at RT.
20. Remove excess liquid.
21. Add Rabbit Anti-BrdU Antibody, for dilution information refer to the #AHP2405 product page (dilute in 1x IF buffer).
22. Incubate for 1 hr at RT.
23. Wash slides 3x with IF buffer.
24. Add a fluorophore conjugated anti-rabbit IgG secondary antibody (such as, Sheep Anti-Rabbit IgG DyLight 549 Conjugated Secondary Antibody, #STAR36D549GA) at a suitable dilution (dilute in IF buffer).
25. Incubate for 1 hr at RT in the dark.
26. (Optional) Add a nuclear counterstain such as PureBlu DAPI (#1351303) and incubate at RT.
27. Wash slides 3x with IF buffer.
28. Wash slides 2x with 1x PBS.
29. Mount coverslip.

Notes

* The acid treatment performed to denature the DNA may affect the immunostaining of some proteins.

Appropriate controls should always be carried out. It may be useful to include an untreated sample.

For further information on controlling your BrdU labeling experiments, refer to the *BrdU in adult neurogenesis research — part 2* blog post at
bio-rad-antibodies.com/blog/brdu-neurogenesis2

Reference

O'Keefe RT et al. (1992). Dynamic organization of DNA replication in mammalian cell nuclei: spatially and temporally defined replication of chromosome-specific alpha-satellite DNA sequences. J Cell Biol 116, 1095-1110.

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