Protocol

BrdU Labeling of HeLa Cells Followed by Immunostaining

ICC

For use with immunocytochemistry tested Rat Anti-BrdU Antibody, clone RF06. 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. 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.
Please also 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)
- Hydrochloric acid (HCl)
- Immunofluorescence buffer (IF buffer) (prepare by adding 0.75 g glycine per 100 ml phosphate buffered saline (PBS))
- Paraformaldehyde (PFA)
- PBS Triton X-100
- Rat Anti-BrdU Antibody clone RF06 (Cat. #MCA6144)
- (Optional) PureBlu DAPI Nuclear Staining Dye (#1351303) and antibody to stain cytoplasmic proteins for example Rabbit Anti-GAPDH Antibody (#AHP1628)

Method

1. Place sterilized glass cover slips into two wells of a 6-well plate. Seed HeLa cells at a density of 1 x 10^4 cells /well in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS into the wells containing cover slips (5 ml of media per well). Allow the cells to settle for 24-48 hr or until approximately 80 % confluent.
2. Prepare 5 ml of BrdU treated medium by diluting 100 mM BrdU stock solution 1:1,000 in DMEM culture medium containing 10% FBS.
3. Prepare 5 ml of DMSO treated medium by diluting DMSO 1:1,000 in DMEM culture medium containing 10% FBS.
4. Remove the culture medium from the cells by pipette. Add the 5 ml of media containing BrdU to the well of cells to be labeled with BrdU, and the 5 ml of media containing DMSO to the well of cells that are to act as your negative control. Incubate BrdU and DMSO treated HeLa cells for 3 hr at 37°C.
5. Wash the cells three times (3x) with 2 ml of 1x PBS/well.
6. Add 1 ml of PBS with 4% PFA/well. Incubate for 15 min at RT.
7. Wash the cells 3x with 2 ml of 1x PBS/well.
8. Add 1 ml of 1% Triton X-100/well. Incubate for 10 min at RT.
9. Wash the cells 3x with 2 ml of 1x PBS/well.
10. Add 1 ml 2 M HCl /well. Incubate for 30 min at RT.
11. Wash the cells 3x with 2 ml of 1x PBS/well.
12. Wash cells once with 2 ml of IF buffer/well.
13. Add 1 ml of IF buffer to each well of cells. Incubate for 30 min at RT. Remove excess liquid by pipetting.
14. Add 1 ml of PBS with 10 % FCS to each well of cells. Incubate for 30 min at RT. Remove liquid by pipetting (do not wash off).
15. Make up 2 ml of primary antibody mixture by adding 4 µg of Rat Anti-BrdU Antibody (#MCA6144) and 4 µg of Rabbit Anti-GAPDH Antibody (#AHP1628) to 2 ml of IF buffer. Add 1ml of primary antibody mixture to the well containing BrdU treated cells and 1 ml of primary antibody mixture to the well containing DMSO treated cells. Incubate at 4°C overnight without agitation.
16. Wash slides 3x with 1 ml of IF buffer/well.
17. Make up 2 ml of secondary antibody mixture by adding 10 µg of Goat Anti-Rat IgG (H/L): TRITC (#305003) and 4 µg of Goat Anti-Rabbit IgG: Dylight 488 Antibody (#STAR36D488GA) to 2 ml of IF buffer. Add 1 ml of secondary antibody mixture to the well containing BrdU treated cells and 1 ml of secondary antibody mixture to the well containing DMSO treated cells. Incubate for 1 hr at RT protected from light and without agitation.
18. Add 1 ml of 0.5 µg/ml of PureBlu DAPI (#1351303) diluted in PBS to each well of cells and incubate at RT for 2 min.

19. Wash cells 3x with 1 ml of IF buffer/well.
20. Wash cells twice in 1 x PBS/well.
21. Remove the coverslips from the 6-well plate and remove excess liquid with tissue. Bond the cover slips to microscope slides with mounting medium. Store at 4°C protected from light until ready to image.

Bio-Rad is a trademark of Bio-Rad Laboratories, Inc. in certain jurisdictions. All trademarks used herein are the property of their respective owner.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad-antibodies.com*