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Monoclonal Antibody to Bromodeoxyuridine / BrDU - Biotin

Catalog No.: SM1667B

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: The immunocytochemical detection of bromodeoxyuridine (BrdU) incorporated into DNA is a powerful tool to study the cytokinetics of normal and neoplastic cells. In vitro or in vivo labeling of tumor cells with the thymidine analogue BrdU and the subsequent detection of incorporated BrdU with specific anti-BrdU monoclonal antibodies is an accurate and comprehensive method to quantitate the degree of DNA-synthesis. BrdU is incorporated into the newly synthezised DNA of S-phase cells may provide an estimate for the fraction of cells in S-phase. Also dynamic proliferative information such as the S-phase transit rate and the potential doubling time can be obtained, by means of bivariate BrdU/DNA flow cytometric analysis.

Host / Isotype: Rat / IgG2a

Clone: BU1/75 (ICR1)

Format:
State: Liquid purified IgG fraction from Tissue Culture Supernatant
Purification: Affinity Chromatography on Protein G
Buffer System: PBS
Preservatives: 0.09% Sodium Azide
Stabilizers: 1% BSA
Label: Biotin

Applications:
Flow Cytometry: Use 20 µl of neat Antibody to label 10^6 cells in 100 µl.
See **Protocol** for more details.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Reacts with BrdU in single stranded DNA, BrdU attached to a protein carrier or free BrdU. The antibody detects nucleated cells in S-phase which have had BrdU incorporated into their DNA. It also reacts with Chlorodeoxyuridine but with reduced staining. The antibody **does not cross** react with Thymidine.
Anti BrdU antibody cross reacts with Chlorodeoxyuridine (CldU) but does not cross react with Thymidine or Iododeoxyuridine (*Aten et al.* 1992). BrdU, IdU and CldU are analogs of Thymidine, they can incorporate into DNA during DNA synthesis replacing Thymidine. Antibody detection of incorporated BrdU in cellular DNA is extensively referenced as an accurate method to monitor cell proliferation in vivo and *in vitro*. In cell proliferation assays BrdU staining is coupled with the use of a dye that binds total DNA such as Propidium Iodide (PI). BrdU can be administered diluted in the culture medium or, *in vivo* via intraperitoneal injection, subcutaneous osmotic pump implants (*Tesfaiqzi et al.* 2004) or in

drinking water (*Moser et al.* 2004).
The BrdU antibody clone BU1/75 (ICR1) has been used to detect CldU to study the speed of DNA replication fork (*Bugler et al.* 2010), in the detection of CldU label retaining stem cells (*Kimoto et al.* 2008) and label retaining neurons (*Murata et al.* 2011).

Storage:

Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings:

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9. Gonzalo-Gobernado, R. et al (2009) Mobilization of neural stem cells and generation of new neurons in 6-OHDA-lesioned rats by intracerebroventricular infusion of liver growth factor. J Histochem Cytochem. 57: 491-502
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12. Bonzo, J.A. et al. (2012) Suppression of Hepatocyte Proliferation by Hepatocyte Nuclear Factor 4a in Adult Mice. J Biol Chem. Jan 12. [Epub ahead of print]
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20. Puverel, S. et al. (2011) RanBPM is essential for mouse spermatogenesis and

oogenesis. Development. 138: 2511-21.
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Protocols:

Flow Cytometry Analysis

Prepare the following solutions before proceeding:
Phosphate buffered saline (PBS)
2N HCl containing 0.5% Triton X-100
PBS containing 0.05% Tween-20
PBS containing 1% BSA (PBS/BSA)
10 mg/ml Propidium iodide (PI)
0.1 M $Na_2B_4O_7$, pH 8.5

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 µmol/L and incubate for 30 min in a CO_2 incubator at 37°C.
2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 min, decant supernatant and resuspend in a miniumum volume of PBS.
3. Add cells slowly into 5 ml of 70% ethanol at -20°C, mixing continuously (vortex preferred). Incubate on ice for 30 min.
4. Centrifuge at 500g for 10 min, decant supernatant, and resuspend cell pellet.
5. Add 2 ml of 2N HCl containing 0.5% Triton X-100 and incubate the cells for 30 min at RT (preferably on a rocking platform).
6. Centrifuge at 500g for 10 min, decant supernatant and resuspend in 3 ml of 0.1 M $Na_2B_4O_7$, pH 8.5.
7. Centrifuge at 500g for 10 min, decant supernatant and resuspend the cells in PBS/BSA + 0.05% Tween-20. Adjust cell concentration to 1 x 10^7/ml.
8. Aliquot 100 ul of cell suspension into required number of 12 x 75 mm tubes.
9. Incubate the cells with the BrdU antibody at the recommended dilution for 30 min at RT.
10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000 rpm for 5 min.
11. If a secondary antibody layer is required then decant the supernatant and incubate the cells with the secondary antibody for 30 min at RT. If no secondary antibody layer is required then proceed to step 13.
12. Wash the cells by repeating step 10.
13. Decant off the supernatant and add 1ml of PBS containing 10 µg/ml PI (dilute the 10 mg/ml solution of PI 1/1000 in a suitable volume of PBS)

14. Analyse cells by Flow Cytometry following the manufacturers instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale.