

## Monoclonal Antibody to Bromodeoxyuridine / BrdU - Purified

**Catalog No.:** SM1667PT

**Quantity:** 20 µg

**Concentration:** 0.5 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 synthesized 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

**Recommended Isotype Controls:** SM15P, SM15PX

**Clone:** BU1/75

**Format:** **State:** Liquid purified IgG fraction  
**Purification:** Affinity Chromatography on Protein G  
**Buffer System:** PBS  
**Preservatives:** 0.09% Sodium Azide

**Applications:** **Flow Cytometry:** Use 20 µl of 1/25-1/200 diluted antibody to label 10<sup>6</sup> cells in 100 µl. Membrane permeabilization is required (See protocol below).  
**Immunohistochemistry on Formalin-Fixed, Paraffin-Embedded Sections:** 1/25-1/200. Denaturation of the DNA is critical for successful staining of BrdU. This can be achieved by exposing cells to heat or acid. For heat-induced epitope retrieval, 10 mM citrate buffer pH 6.0 is recommended. Alternatively, a 30 min incubation in 2M HCl can be performed. The HCl must then be neutralized for 2 min with 0.1 M Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>. Pre-treatment of tissues with proteinase K should be avoided.  
**Immunocytochemistry:** 1/50-1/100.  
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:** This antibody reacts with BrdU in single stranded DNA, BrdU attached to a protein carrier or free BrdU.  
The antibody detects nucleated cells in S-phase with have had BrdU incorporated into their DNA.

It also reacts with Chlorodeoxyuridine but with reduced staining.  
The antibody does not cross react with Thymidine.

**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.

**Product Citations:****Purchased from Acris:**

1. Sonal, Sidhaye J, Phatak M, Banerjee S, Mulay A, Deshpande O, et al. Myosin Vb mediated plasma membrane homeostasis regulates peridermal cell size and maintains tissue homeostasis in the zebrafish epidermis. *PLoS Genet.* 2014 Sep 18;10(9):e1004614. doi: 10.1371/journal.pgen.1004614. eCollection 2014 Sep. PubMed PMID: 25233349.

**General Readings:**

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2. Ghiringelli, F. et al. (2005) Tumor cells convert immature myeloid dendritic cells into TGF-beta-secreting cells inducing CD4+CD25+ regulatory T cell proliferation. *J. Exp. Med.* 202: 919-929.
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4. Das, G. et al. (2009) Cyclin D1 fine-tunes the neurogenic output of embryonic retinal progenitor cells. *Neural Dev.* 4: 15.
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15. Kim, T.H. et al. (2011) Genetic evidence that intestinal notch functions vary regionally and operate through a common mechanism of math1 repression. *J Biol Chem.* Jan 31. [Epub ahead of print]
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17. Liu, M.T. et al. (2009) 5-HT4 receptor-mediated neuroprotection and neurogenesis in the enteric nervous system of adult mice. *J Neurosci.* 29: 9683-99.
18. Lundgren, O. et al. (2011) Intestinal epithelial stem/progenitor cells are controlled by mucosal afferent nerves. *PLoS One.* 6: e16295.
19. Muja, N. et al. (2011) Neural precursors exhibit distinctly different patterns of cell

**For research and in vitro use only. Not for diagnostic or therapeutic work.**

Material Safety Datasheets are available at [www.acris-antibodies.com](http://www.acris-antibodies.com) or on request.

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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  $\text{Na}_2\text{B}_4\text{O}_7$ , pH 8.5

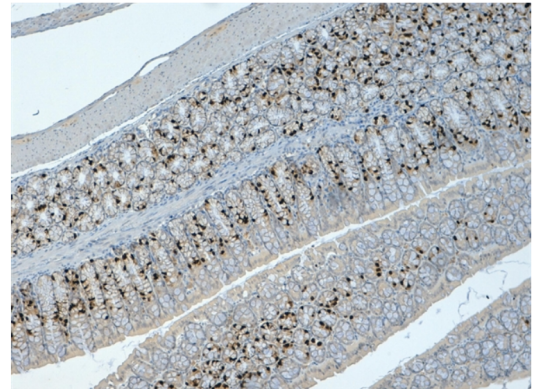
As a Positive Control, BrdU labelled cells maybe obtained from Phoenix Flow Systems (<http://www.phnxflow.com>), catalogue number ACNC12.

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10  $\mu\text{mol/L}$  and incubate for 30 min in a  $\text{CO}_2$  incubator at 37°C.
2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 min, decant supernatant and resuspend in a minimum 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  $\text{Na}_2\text{B}_4\text{O}_7$ , 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  $1 \times 10^7/\text{ml}$ .
8. Aliquot 100  $\mu\text{l}$  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  $\mu\text{g/ml}$  PI (dilute the 10  $\mu\text{g/ml}$  solution of PI 1/1000 in a suitable volume of PBS)

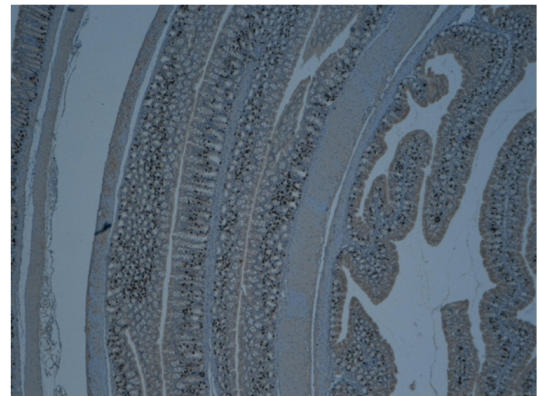
14. Analyse cells by Flow Cytometry following the manufacturers instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale.

**Pictures:**

Formalin-Fixed, Paraffin-Embedded  
Mouse colon stained with Rat anti BrdU  
Antibody (Clone BU1/75): low power



Formalin-Fixed, Paraffin-Embedded  
Mouse colon stained with Rat anti BrdU  
Antibody Cat.-No SM1667PS  
(Clone BU1/75): low power.



HL-60 cells were pulse labeled with BrdU  
for 45 minutes prior to harvesting and  
then incubated with primary antibody Rat  
anti BrdU clone BU1/75 diluted 1/100  
followed by Rabbit anti Rat IgG  
secondary antibody (Cat.-No  
SP1021F) FITC-conjugated, diluted 1/200

