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SM1667PS Monoclonal Antibody to Bromodeoxyuridine / BrDU - Purified

Quantity: 0.1 mg

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 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

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 10^6 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 $Na_2B_4O_7$. 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 antibdoy reacts with BrdU in sigle 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 Chlorodeocyuridine 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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14. Grotek, B. et al. (2013) Notch signaling coordinates cellular proliferation with differentiation during zebrafish fin regeneration. Development. 140: 1412-23.
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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

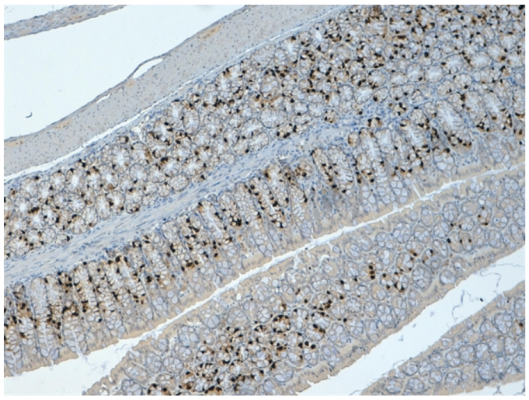
As a Positive Control, BrdU labelled cells maybe obtained from Phoenix Flow Systems (http://www.phnxflow.com), catalogue number ACNC12.

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 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.

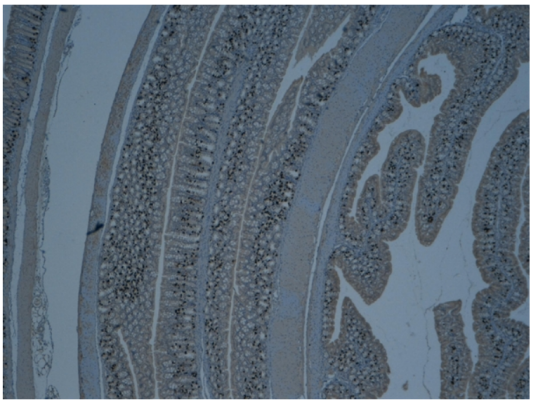
8. Aliquot 100 ul of cell suspension into required number of 12 x 75 mm tubes.
9. Incubate the cells with the BrdU antibody at the recommended dilution for 30 min at RT.
10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000 rpm for 5 min.
11. If a secondary antibody layer is required then decant the 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.

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