Product Datasheet

Bromodeoxyuridine/BrdU Antibody (Bu20a)
NB100-64345

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Publications: 3

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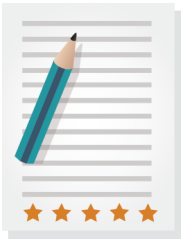

NB100-64345

Bromodeoxyuridine/BrdU Antibody (Bu20a)

Product Information	
Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	Bu20a
Preservative	0.09% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Species	Non-species specific
Reactivity Notes	Reacts with Chemical
Marker	Proliferation Marker
Specificity/Sensitivity	NB100-64345 recognizes the thymidine analogue bromodoxyuridine (BrdU), which can be incorporated into DNA during S-phase of the cell cycle. The BU20a antibody is suitable for detecting incorporated BrdU in a wide variety of cell types and is suitable for use on tissue sections in double-labelling techniques.
Immunogen	Bromodeoxyuridine conjugated to BSA.

Product Application Details	
Applications	Flow Cytometry, Immunohistochemistry, Immunohistochemistry-Frozen, Immunohistochemistry-Paraffin
Recommended Dilutions	Flow Cytometry 1:25-1:100, Immunohistochemistry 1:10-1:500, Immunohistochemistry-Paraffin 1:10-1:500, Immunohistochemistry-Frozen 1:10-1:500
Application Notes	For IHC-Frozen: Frozen sections and cell preparations: Acetone-fixed, frozen sections and cell preparations are suitable for staining with clone Bu20a. To survey brain structure overall, use AChE histochemistry, a standard way to identify brain subdivisions. IHC- Formalin-fixed paraffin-embedded tissue sections: Clone Bu20a can be used for labeling paraffin-embedded tissue sections fixed in formalin. 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, 10mM citrate buffer pH6.0 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 Na2B4O7. Pretreatment of tissues with proteinase K should be avoided. Flow Cytometry: Use 10ul of the suggested working dilution to label 1x106 cells in 100ul.

Publications

Caronia, G et al. Bone morphogenetic protein signaling in the developing telencephalon controls formation of the hippocampal dentate gyrus and modifies fear-related behavior. J Neurosci 30: 6291-301. 2010-01-01 [PMID: 20445055] (IF/IHC)

Magaud JP, Sargent I, Clarke PJ et al. Double Immunocytochemical labelling of cell and tissue samples with monoclonal anti-bromodeoxyuridine. J Histochem Cytochem 37:1517 - 1527. 1989-01-01 [PMID: 2476478]

Innis, SM et al. Perinatal Lipid Nutrition Alters Early Intestinal Development and Programs the Response to Experimental Colitis in Young Adult Rats. Am J Physiol Gastrointest Liver Physiol. 2010-09-23 [PMID: 20864654]

Procedures

FLOW CYTOMETRY (NB100-64345)
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)
10mg/ml Propidium iodide (PI)
0.1M $Na_2B_4O_7$, pH 8.5

1. Add BrdU to the cell suspension in culture medium to a final concentration of 10 umol/L and incubate for 30 minutes in a CO_2 incubator at 37 degrees C.
2. Wash cells twice with PBS/BSA by centrifuging at 500g for 10 minutes, decant supernatant and resuspend in a minimum volume of PBS.
3. Add cells slowly into 5ml of 70% ethanol at -20 degrees C, mixing continuously (vortex preferred). Incubate on ice for 30 minutes.
4. Centrifuge at 500g for 10 minutes, decant supernatant, and resuspend cell pellet.
5. Add 2ml of 2N HCl containing 0.5% Triton X-100 and incubate the cells for 30 minutes at room temperature (preferably on a rocking platform).
6. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend in 3 ml of 0.1M $Na_2B_4O_7$, pH 8.5
7. Centrifuge at 500g for 10 minutes, decant supernatant and resuspend the cells in PBS/BSA + 0.05% Tween-20. Adjust cell concentration to 1 x 107/ml.
8. Aliquot 100ul of cell suspension into required number of 12 x 75mm tubes.
9. Incubate the cells with the BrdU antibody at the recommended dilution for 45 minutes at room temperature or overnight at 4 degrees C.
10. Add 2 ml of PBS/BSA and centrifuge the cells at 1000rpm for 5 minutes.
11. If a secondary antibody layer is required then decant the supernatant and incubate the cells with the secondary antibody for 30 minutes at room temperature. If no secondary antibody layer is required then proceed to step 13.
12. Wash the cells after the secondary antibody layer by repeating step 10.
13. Decant the supernatant and add 1ml of PBS containing 10ug/ml PI (Dilute the 10mg/ml solution of PI 1/1000 in a suitable volume of PBS).
14. Analyze cells by flow cytometry following the manufacturer's instructions. The PI should be read on the appropriate channel set to the Peak/Area and not log scale

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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