Product Datasheet

Bromodeoxyuridine/BrdU Antibody (BU1/75 (ICR1)) [FITC] NB600-720

Unit Size: 0.125 ml

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

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Publications: 1

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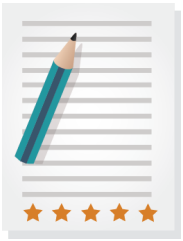
Updated 3/30/2022 v.20.1



NB600-720

Bromodeoxyuridine/BrdU Antibody (BU1/75 (ICR1)) [FITC]

Product Information	
Unit Size	0.125 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	BU1/75 (ICR1)
Preservative	0.01% Thimerosal, 0.05% Sodium Azide
Isotype	IgG2a
Conjugate	FITC
Purity	Protein G purified
Buffer	10 mM Sodium Phophsate (pH 7.6), 0.25 M NaCl, and 15 mg/ml BSA

Product Description	
Host	Rat
Species	Human, Mouse, Rat, Non-species specific, Drosophila, Monkey
Reactivity Notes	Monkey reactivity reported in (PMID: 28566378). Rat reactivity reported in (PMID:23966683). Drosophila reactivity reported in (PMID:22438819).
Marker	Proliferation Marker
Specificity/Sensitivity	This reacts with BrdU in single stranded DNA, BrdU attached to a protein carrier or free BrdU. It detects nucleated cells in S-Phase which have had BrdU incorporated into their DNA. Also reacts weakly with chlorodeoxyuridine, but does not cross-react with thymidine or iododeoxyuridine.
Immunogen	Made to Chemical BrdU

Product Application Details	
Applications	Flow Cytometry
Recommended Dilutions	Flow Cytometry Neat-1:20

Publications

He X, Smith SE, Chen S et al. Tumor-initiating stem cell shapes its microenvironment into an immunosuppressive barrier and pro-tumorigenic niche Cell reports 2021-09-07 [PMID: 34496236]

Procedures

Immunocytochemistry/Immunofluorescence Protocol for BrdU Antibody (NB600-720)
Staining of DNA-synthesizing cells with BrdU-FITC

Incorporation of 5-Bromo-2-Deoxyuridine into DNA

5'??-Bromo-2'??-deoxyuridine (BrdU) is incorporated into the DNA of S-phase (DNA-synthesising) cells.

BrdU is simply added to the culture medium at a final concentration of 10-20mM together with 2'??-deoxycytidine (20-50mM). Short pulses (i.e. brief presence of BrdU in culture medium) of 10 min or less can be detected. However, for routine work pulses of 1-3 h are recommended.

Direct Immunofluorescence Staining

a) Monolayer Cells

1. Wash cells, grown on slides or cover slips, twice with PBS.
2. Fix cells with cold methanol for 20 minutes at -20C (at this stage cells may be kept for 1 month at -20C).
3. Pre-wet dry cover slips by a short incubation in PBS.
4. Denature DNA to its single-stranded form by two consecutive
treatments with 4M HCI for 15 minutes each.
5. Wash 3 times with PBS.
6. Incubate with the anti-BrdU-FITC-conjugate (30 minutes, room temperature humidity chamber).
7. Wash twice with PBS.
8. Mount dry samples with standard mounting medium and evaluate the results by fluorescence microscopy.

b) Suspension cells

1. Wash cells twice with PBS (250g, 7 minutes).
2. Resuspend cells in 3 vol PBS (0oC) and fix cells by adding 9 vol methanol (-20oC) whilst mixing the cell suspension. Incubate for 20 minutes.
3. Denature cells by adding one equal volume of 4M HCI to fixed cell suspension (20 minutes room temperature).
4. Pellet cells and resuspend the pellet in 4 M HCI, incubate for 15 minutes.
5. Carefully remove HCI by three washes with PBS (5000g, 7 minutes).
6. Resuspend cell pellet in ready to use staining solution (ca. 25ml/106 cells).
7. Wash twice with PBS.
8. Mount dry samples with standard mounting medium and evaluate the results by fluorescence microscopy.

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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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