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Monoclonal Antibody to Bromodeoxyuridine (BrDU) - Purified

Catalog No.: BM5532P

Quantity: 0.1 mg

Background: Bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU) is a synthetic nucleoside which is an analogue of thymidine. BrdU is commonly used in the detection of proliferating cells in living tissues. BrdU can be incorporated into the newly synthesized DNA of replicating cells (during the S phase of the cell cycle), substituting for thymidine during DNA replication. Antibodies specific to BrdU can then be used to detect the incorporated chemical; thus indicating cells that were actively replicating their DNA. Binding of the antibody requires denaturation of the DNA by heat or acid.

Host / Isotype: Mouse / IgG2a

Clone: Bu5.1

Immunogen: Bromodeoxyuridine

Format:
State: Lyophilized purified IgG fraction
Purification: Protein A Affinity Chromatography
Buffer System: PBS buffer, pH 7.4 containing 0.09% Sodium Azide as preservative and 0.5% BSA as stabilizer
Reconstitution: Restore in 1 ml dist. water

Applications: Detection of Bromodeoxyuridine incorporation in tissue and cell culture. (See protocols on page 2.
Working Dilution: 1/10.
Incubation time: 30 min at RT.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: The antibody is specific for Bromodesoxyuridine and does not cross-react with Thymidine. It is a valuable tool for detection of S-phase (DNA-synthesizing cells) in cell ad tissue culture. 3H-thymidine labelling techniques can be avoided.

Storage: Prior to reconstitution store at 2-8°C.
Following reconstitution store the antibody -20°C.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References:
1. Hosihino, T., Nagashima, T., Murovic, J., Levin, E.L., Levin V.A. and Rupp, St.M.: Cell kinetic studies of in situ human brain tumors with bromodeoxyuridine. Cytometrie 6, 627-632 (1985). Jirikowski G.F., Ramalho-Ortigao F., Kesse 2. K.W. and Bloom F.E.: In situ hybridization of semithin Epon sections with BrdU labelled oligonucleotide probes. Histochemistry 94, 187-190 (1990)

Protocols:

Staining of BrdU-labelled DNA in Proliferating Cells with Mab BU 5.1

Incorporation of 5'-Bromo-2'-Desoxyuridin (BrdU) into DNA
5'-bromo-2'-desoxyuridin (BrdU) is incorporated into the DNA of S-Phase (DNA-synthesizing) cells. [Cat.-No. BM5532F(contains Evans Blue), Cat.-No. BM5532P] With Mab BU 5.1 the proportion of cells in the S-Phase of the cell cycle can be easily identified because this Mab is specific for BrdU-substituted DNA.
BrdU is added to the culture medium at a final concentration of 10-20 µM together with 2'-desoxycytidine (20-50 µM).
For routine work pulses of 1-3 h are recommended.
Short pulses (i.e. brief incubation of BrdU within the culture medium of 10 min are detectable). I. Indirect

I. Indirect Immunofluorescence Microscopy

1. Monolayer Cells
1. Wash cells, grown on slides or cover slips, twice with PBS.
2. Fix cells with cold 70% ethanol for 20 min (at this stage, cells may be kept for 1 month at -20°C).
3. Denature DNA with 2.5 N HCl for 20 min.
4. Wash 3x with PBS.
5. Incubate with Mab BU 5.1 for 30 min at room temperature.
6. Wash 3x with PBS.
7. Add Fluorochrome-conjugated second antibody (e.g. goat anti-mouse FITC conjugate) in appropriate dilution (incubate for 30 min).
8. Wash 3x with PBS.
9. Mount dry samples with standard mounting medium and evaluate with fluorescence microscope.

2. Suspension Cells
1. Wash and spin cells twice with PBS (250 g, 7 min).
2. Resuspend cells in 3 vol PBS (0°C) and fix cells by adding 7 vol 96% ethanol (0°C) whilst mixing the cell suspension. Incubate for 20 min.
3. Denature cells by adding one equal volume of 4 N HCl to fixed cell suspension (20 min; room temperature).
4. Carefully wash and spin three times with PBS to remove HCl (250x g, 7 min).
5. Staining of BrdU-substituted DNA is performed as described above under I.1.(steps 5-9). The washing steps may be reduced in order to minimize cell loss during centrifugation.

II. Direct Immunofluorescence Microscopy

Mab BU 5.1-FITC (Cat.-No. BM5532F) is supplied in ready-to-use form. Cells processed according to steps 1-4 of I.1. a. or I.2. above are stained by adding 25 µl of the BU 5.1-FITC conjugate. After 30 min incubation, the cells are washed twice, embedded and are then ready for examination.