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ProductInformation

MONOCLONAL ANTI-BRDU
CLONE BU 33
Immunohistology Grade
Mouse Ascites Fluid

Product Number **B 2531**
Storage Temperature –20 °C

Product Description

Monoclonal Anti-BrdU (Immunohistology Grade) (mouse IgG1 isotype) is derived from the BU-33 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a BALB/c mouse immunized with BromodeoxyUridine conjugated to KLH. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

A broad range of biological and biomedical investigations depend on the ability to distinguish DNA synthesizing cells. Until recently, these studies were often limited by the traditional measurement techniques that are based on the determination of incorporated radioactive DNA precursors such as tritiated thymidine. BromodeoxyUridine (5-Bromo-2-DeoxyUridine, BrdU) is a pyrimidine analogue of thymidine, selectively incorporated into cell DNA at the S phase of the cell cycle. The use of BrdU as a thymidine analogue has made the identification of DNA synthesis in suspensions of cells, cell smears and tissue sections possible. The application of monoclonal antibodies which react specifically with BrdU[1,2,3] for detection of DNA replication in lymphoid cells[4] and other normal or pathological preparations,[5] following in vivo or in vitro BrdU labeling, is extensively documented in the biomedical literature. Monoclonal antibodies against BrdU have also proven valuable for studies on cell cycle kinetics, repair synthesis of DNA, demonstration of sister chromatid exchange and assessment of cell proliferation in the presence of growth factors or cytotoxic drugs.

Monoclonal Anti-BrdU (Immunohistology Grade) reacts specifically with BrdU (BromodeoxyUridine) incorporated into DNA, or coupled to a protein carrier. It recognizes BrdU in the nuclei of formalin-fixed, paraffin-embedded tissue sections of animals treated with an in vivo administration of BrdU.

Monoclonal Anti-BrdU (Immunohistology Grade) may be used for the detection of BrdU-labeled preparations using various immunocytochemical and immunohistochemical assays.

Reagents

The product is provided as ascites fluid with 15 mM sodium azide as preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Product Profile

The minimum antibody titer of 1:1,000 was determined by immuno-peroxidase labeling of formalin-fixed, paraffin-embedded sections of intestine from mouse or rat, treated in vivo with BrdU.

Storage/Stability

Store at 2-8 °C for up to one month.

For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure: BrdU Staining in Formalin-Fixed Paraffin-Embedded Tissues with Monoclonal Anti- BrdU

A. Materials:

1. Formalin-fixed paraffin-embedded sections (4-6 µ) of mouse or rat intestines prelabeled in vivo (intraperitoneal) with 1 hour pulse of BrdU (Product No. B 5002, 50 mg/kg).
2. Diluent: Phosphate buffered saline pH 7.2-7.4 containing 1% BSA, 0.05% Tween 20 and 0.1% NaN_3.

3. Blocking Solution: 5% normal goat serum (Product No.G 9023 in Diluent).
4. 2N HCl in distilled water.
5. 0.4% (w/v) pepsin (Product No. P 7012) in 0.01N HCl or 0.1% (w/v) Trypsin (Product No. T 7409) in PBS.
6. 3% Hydrogen peroxide (Product No. H 6520).
7. Monoclonal Anti-BrdU in diluent.
8. Mouse ExtrAvidin Staining Kit components (Product Code EXTRA-2).
9. AEC staining kit (Product Code AEC-101).
10. Glycerol gelatin (Product Code GG1).

Warning: BrdU is toxic and a possible teratogen, possible mutagen. Avoid contact with the material. Use gloves and eye/face protection. Please refer to the MSDS for BrdU.

B. Method:
1. Deparaffinize and rehydrate sections following the Immunohistology Procedure of the Mouse ExtrAvidin Staining kit.
2. Rinse in PBS.
3. Block endogenous peroxidase activity with 3% H_2O_2 for 10 minutes at 37 °C.
4. Rinse in PBS.
5. DNA Denaturation: Place sections in 2N HCl, 30 minutes at 37 °C. Rinse thoroughly in PBS (prerinsing in 0.1M $Na_2B_4O_7$ (Borax) is optional).
6. Enzymatic Pretreatment: Apply 100 µl of prewarmed pepsin or trypsin solutions onto sections. Incubate 30 minutes (with pepsin or 20 minutes with trypsin) at 37 °C. Rinse in PBS.
7. Blocking: Apply 100 µl Blocking Solution for 15 minutes at 37 °C. Tap excess solution. Do not wash.
8. Apply 100 µl Monoclonal Anti-BrdU diluted in Diluent. Incubate 2 hours at 37 °C. Rinse in PBS.
9. Proceed with the biotinylated second antibody and ExtrAvidin peroxidase following the Immunohistology Procedure of the Mouse ExtrAvidin Staining kit.
10. Develop AEC color following the directions of the kit. Do not counterstain.
11. Apply coverslip with liquid glycerol gelatin.

C. Results:
1. S-phase nuclei in villi stain red. Mouse or rat plasma cells in lamina propria may show cytoplasmic staining (mouse or rat immunoglobulin). In sections of unlabeled intestine (BrdU negative sections) or those incubated with PBS or with irrelevant primary antibody no nuclear staining is observed.
2. In rat sections, use of Biotinylated Goat Anti Mouse Fab adsorbed on human I.G. and rat serum proteins (Product No. B 0529) at 20 µg/ml eliminates plasma cell staining.

References

1. Gratzner, H., Science, 218, 474 (1982).
2. Gray, J., (Editor), Special Issue: Monoclonal Antibodies Against Bromodeoxyuridine, Cytometry, Vol. 6 (6) (1985).
3. Wilson, G. Cell kinetic studies using a monoclonal antibody to bromodeoxyuridine, Methods in Molecular Biology 10: 387-398 (1992).
4. Nakamura, S., et al., Oncology, 48, 285 (1991).
5. Meyer, J., et al., J. Histochem. Cytochem., 37, 1449 (1989).

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