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Product Information

Anti-BrdU antibody, Mouse monoclonal
clone BU-33, purified from hybridoma cell culture
Immunohistology Grade

Product Number **B8434**

Product Description

Anti-BrdU antibody, Mouse monoclonal (mouse IgG1) is derived from the BU-33 hybridoma produced by the fusion of murine myeloma cells and splenocytes from BALB/c mouse immunized with bromodeoxyuridine (BrdU) conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Number ISO2).

Anti-BrdU antibody, Mouse monoclonal reacts specifically with 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 may be used for the detection of BrdU-labeled preparations using various immunocytochemical and immuno-histochemical assays.[1-4]

Broad ranges of biological and biomedical investigations depend on the ability to distinguish DNA synthesizing cells. The determination of incorporated radioactive DNA precursors such as tritiated thymidine is one of the known methods but not the most desired one.

Bromodeoxyuridine (5-Bromo-2-Deoxyuridine, BrdU) is a pyrimidine analogue of thymidine that is 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 cell suspensions, cell smears, and tissue sections possible. The application of monoclonal antibodies which react specifically with BrdU [5-7] for the detection of DNA replication in lymphoid cells[8] and other normal or pathological preparations,[9] following *in vivo* or *in vitro* BrdU labeling, is extensively documented in the biomedical literature. Monoclonal antibodies against BrdU have also proven valuable for studying cell cycle kinetics and DNA repair synthesis, and also for assessing cell proliferation in the presence of growth factors or cytotoxic drugs and demonstrating sister chromatid exchange.

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~1.5 mg/ml

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at −20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

Immunohistochemistry: a working antibody concentration of 1-2 μg/ml is recommended using immunoperoxidase labeled formalin-fixed, paraffin-embedded sections of intestine from mice or rats, treated *in vivo* with BrdU.

Note: In order to obtain the best results in various techniques and preparations, it is recommended to determine the optimal working dilution by titration.

Procedure - Immunohistochemistry

Materials:

1. Formalin-fixed, paraffin-embedded sections (4-6 μm) of mouse or rat intestines prelabeled *in vivo* (intraperitoneal) with 1 hour pulse of BrdU, Product Number B5002, 50 mg/kg
2. Diluent: phosphate buffered saline, pH 7.2-7.4, containing 1% BSA, 0.5% TWEEN® 20, and 0.1% sodium azide
3. Blocking solution: 5% normal goat serum, Product Number G9023, in diluent
4. 2 M HCl in distilled water
5. 0.4% (w/v) pepsin, Product Number P7012, in 0.01 M HCl or 0.1% (w/v) trypsin, Product Number T7409, in PBS
6. 3% hydrogen peroxide
7. Monoclonal Anti-BrdU in diluent
8. Mouse ExtrAvidin® Staining Kit Components, Product Number EXTRA2
9. AEC staining kit, Product Number AEC101
10. Glycerol gelatin, Product Number GG1

Method:

1. Deparaffinize and rehydrate sections following the immunohistology procedure in the mouse Extravidin staining kit.
2. Rinse in PBS for 15 minutes at room temperature.
3. Block endogenous peroxidase activity with 3% H_2O_2 for 10 minutes at 37 °C.
4. Rinse in PBS for 5 minutes at room temperature.
5. DNA Denaturation: Place sections in 2 M HCl for 30 minutes at 37 °C. Rinse thoroughly in PBS (prerinsing in 0.1 M $Na_2B_4O_7$ (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 off excess solution. Do not wash.
8. Apply 100 μl of Monoclonal Anti-BrdU diluted in diluent. Incubate 2 hours at 37 °C. Rinse in PBS three times for 5 minutes each.
9. Proceed with the biotinylated second antibody and ExtrAvidin peroxidase using the immunohistology procedure with the mouse ExtrAvidin Staining Kit.
10. Develop the AEC color using the directions in the kit. Do not counterstain.
11. Apply coverslip with liquid glycerol gelatin.

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, the use of biotinylated goat anti-mouse Fab adsorbed on human immunoglobulins and rat serum proteins (Product Number B0529) at 20 μg/ml, eliminates plasma cell staining.

References

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