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Monoclonal Antibody to Bromodeoxyuridine (BrDU)

Catalog No.: BM6048

Quantity: 1 ml

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 / IgG1

Clone: IIB5

Immunogen: BrdU conjugated to BSA.

Format: **State:** Liquid Ascites

Applications:
Flow Cytometry.
In *situ*-hybridization.
Immunohistochemistry on Frozen Sections.
Immunohistochemistry on Paraffin Sections: Proteolytic treatment with Pepsin is required (see Protocol).
Recommended Dilutions: 1/10
Incubation Time: 1h at RT.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody clone *IIB51* reacts with BrdU in denatured (single-stranded) DNA).
The antibody is cross-reactive with Iododeoxyuridine.
It can be used for:
1. Radioimmunocytochemical detection of circulating levels of BrdU.
2. Detection of S-phase cells in tissue sections by immunoper-oxidase or immunofluorescence method.
3. Detection of S-phase cells in cell suspension.
4. Determination of the percentage of profilerating cells by Flow Cytometry.
5. Quantitative determination of the number of various phases of the cell cycle by dual parameter flow-cytometrical analysis.

Storage: Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General References:
1. Schutte B et al. Effect of Tissue Fixation on Anti-Bromodeoxyuridine Immunohistochemistry. The Journal of Immunohistochemistry and Cytochemistry, Vol 35,

For research and in vitro use only. Not for diagnostic or therapeutic work.
Material Safety Datasheets are available at www.acris-antibodies.com or on request.

Antibody Hotline - Technical Questions - Antibody Location Service
Free Call: 0800-2274746 (Germany only) - www.acris-antibodies.com

11:1343-1345 (1987)
2. Schutte B et al. Studies with Anti-Bromodeoxyuridine Antibodies: II. Simultaneous Immunocytochemical Detection of Antigen Expression and DNA Synthesis by in Vivo Labeling of Mouse Intestinal Mucosa. The Journal of Histochemistry and Cytochemistry, Vol 35, 3:371-374 (1987)
3. Schutte B et al: Studies with Anti-Bromodeoxyuridine Antibodies: I An improved method for the immunocytochemical detection of BrdfU labeled nuclei using flow cytometry. Cytometry 8:372-376 (1987)

Protocols:

The Use of Bromodeoxyuridine and Anti-Bbromodeoxyuridine in the Detection of Cell Proliferation Activity (Labeling and Detection Methods)

1. Introduction

The proliferative activity of cells is estimated by measuring the labeling-index (L.I.). The L.I. is defined as the fraction of cells in S-phase at the moment of labeling. In the past the L.I. was measured by incorporation of radioactive labeled thymidine into the DNA. With the introduction of immunohistochemically detectable nucleotides a new non-radioactive method for the estimation of the L.I. has become available. The method is based upon the incorporation of bromodeoxyuridine (BrdU), a thymidine analogue, into reduplicating DNA. With an antibody directed against bromodeoxyuridine (BrdU) the labelled cells can be immunocytochemically detected. Especially on tissue sections this method has many advantages. Measurement of the proliferative activity of e.g. tumour cells is now within the reach of each laboratory.

2. Labeling with bromodeoxyuridine

a) *In vitro* labelling: Cell cultures.
Addition of BrdU (10 µM final concentration) to the cell culture medium. After 10 to 30 minutes the cells are harvested and fixed in the appropriate fixative.
b) In vivo labelling.
Animals are injected intraperitoneally with 5-50 mg BrdU/kg bodyweight. After 1h the animals are sacrificed, the organs
removed, and fixed or snap frozen.
c) *Ex vivo* labeling.
i) 'Single' cell biopsies.
- Aspiration biopsies e.g.: Bone marrow
- Brush preparations e.g.: Lung
- Biopsies are transferred immediately to a cell culture tube (15 ml) containing 10 ml medium pre-incubated at 37°C.
Medium: RPMI 1640 (Hepes buffered)
10% Foetal calf serum
10 µM BrdU
Incubation one hour at 37°C. After labeling transfer biopsy to the appropriate fixative.
ii) Solid tissue biopsies.
Small biopsies, 1-3 mm^3, are taken and immediately transferred to a cell culture tube (15 ml) containing 10 ml medium pre-incubated at 37°C. (see 2.C1). Add three drops of 30% H_2O_2, and firmly close screw cap. Incubate one hour
at 37°C. After incubation, transfer biopsy to the appropriate fixative.

3. Fixation of biopsies

Options: - Frozen sections
- Cytospin preparations
- Flow cytometry
- Paraffin sections

Frozen Sections: After labeling with BrdU, specimens are snap frozen in isopentane quenched in liquid nitrogen. Sections (2-5 µm) are cut in a cryostat at -20°C, and mounted on clean glass slides. After fixation in acetone, for 10 min. at -20°C, sections can be stored at -20°C or below.
Cytospin preparations: After labelling with BrdU, cells are fixed in acetone for 10 min. at -20°C. Rinse 2 times in PBS. Transfer cells to PBS containing 10 mg/ml BSA. Cytocentrifuge and allow slides to dry at RT.
Flow Cytometry: After labeling with BrdU, biopsies are fixed in 70% ethanol. Biopsies can be stored in ethanol at 2-8°C protected from light.
Paraffin Sections: After labelling with BrdU, biopsies are fixed in 70% ethanol for 3 to 24 hours. Transfer sections through a graded alcohol series
and embed in paraffin. Alternatively, biopsies can be fixed in a cross-linking fixative, e.g. 10% buffered formalin. After fixation is completed (maximum fixation time 24 hours), rinse in tap water and transfer biopsies through a graded alcohol series to paraffin.
Sections (2-5 µm) should be stretched on a water bath, and adhered to
Chrome-Alum-Gelatine coated glass slides by incubation overnight at 37°C.

4. Immunocytochemical detection of BrdU labeled DNA

a) Frozen Sections and Cytospin preparations:
- Rinse in PBS 2x5 min.
- Incubate in 2 M HCl for 30 min. at 37°C.
- Rinse in 0.1 M $Na_2B_4O_7$, pH 8.5, 2 x 5 min.
- Incubate in 100 µl 1:10 diluted anti-BrdU* for 60 min. at 37°C
- Rinse in PBS 2 x 5 min.
- Incubate with conjugated RAM Ig* 30 min.
- Rinse in PBSDehydrate and mount with mounting medium.

*Optimal dilution should be tested by serial dilution
- Substrate reaction

Counterstaining
Dehydrate and mount with mounting medium.
*Optimal dilution should be tested by serial dilution

b) Flow Cytometry

Approximately 2 x 10e6 ethanol fixed cells are washed twice in PBS
- Re-suspend the pellet in 2 ml pepsin solution (0.4 mg/ml in 0.1 M HCl) and incubate for 30 min. at RT.
- Rinse in PBS 2 x 5 min.
- Re-suspend the pellet in 2 ml 2 M HCl and incubate for 30 min. at 37°C.
- Rinse in 2 ml 0.1 M $Na_2B_4O_7$, 2 x 5 min.
- Rinse in PBT (PBS plus BSA 1 mg/ml, 0.05% Tween 20, pH 7.4).
- Incubate the pellet in 100 µl 1:10 diluted anti-BrdU for 60 min. at RT.
- Rinse in PBT 2 x 5 min.
- Incubate the pellet in 100 µl appropriately diluted FITC conjugated RAM-Ig for 60 min. at room temperature.
- Rinse in PBT 2 x 5 min.
- Re-suspend the pellet in PBS containing 10 µg/ml propidium iodide and 0.1 mg/ml RNase.

c) Paraffin Sections

i) Ethanol fixed biopsies.
- Clear sections of paraffin.
- Block endogenous peroxidase activity with 0.3% H_2O_2 in 50% methanol for 20 min.
- Rinse in PBS 2 x 5 min.
- Incubate in 2 M HCl for 30 min. at 37°C.
- Rinse in 0.1 M $Na_2B_4O_7$, pH 8.5, 2 x 5 min.
- Rinse in PBS 2 x 5 min.

- Incubate in 100 µl 1:10 diluted anti-BrdU* for 60 min. at 37°C
- Rinse in PBS 2 x 5 min.
- Incubate in 100 µl appropriately diluted peroxidase conjugated RAM-Ig for 60 min. at room temperature.
- Rinse in PBS 2 x 5 min.
- Incubate in diaminobenzidine (DAB) solution: (0.5 mg/ml 3,3 diaminobenzidine and 0.01% H_2O_2 in 0.05 M Tris-HCl pH 7.6 for 10 min.
- Rinse in dist. water. Counterstain, dehydrate and mount with mounting medium.

ii) Formalin fixed biopsies.
- Clear of paraffin and block endogenous peroxidase (see 4.c.i).
- Rinse in PBS 2 x 5 min.
- Incubate with pepsin solution (0.4 mg/ml in 0.1 M HCl) for 30 min. at room temperature.
- Rinse in PBS
- Continue with 2 M HCl incubation as described under 4.c.i.s

Used abbreviations

PBS: Phosphate Buffered Saline
PBT: PBS + BSA + Tween 20
BSA: Bovine Serum Albumin
RAM: Rabbit-Anti-Mouse