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Not for use in diagnostic procedures.

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MONOCLONAL ANTIBODY

Anti-Bromodeoxyuridine mAb

Code No.	Clone	Subclass	Quantity	Concentration
MI-11-3	2B1	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: BrdU (5-Bromo-2-Deoxyuridine) is a derivative of uridine that can substitute for thymidine during DNA synthesis. The detection of BrdU incorporation into DNA is a common method to quantify newly synthesized DNA and to identify cells in the S-phase of the cell cycle. BrdU incorporation is frequently used in proliferation assays to study DNA repair, sister chromatid exchange, and the cytokinetics of normal and neoplastic cells.

SOURCE: This antibody was purified from hybridoma (clone 2B1) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with 5-iodouridine-Ovalbumin.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with bromodeoxyuridine incorporated in the nuclei in Raji cells on Flow cytometry and it reacts with iododeoxyuridine, iodouridine, chlorodeoxyuridine and bromouridine, but does not react with thymidine on Flow cytometry and Immunohistochemistry.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation: Reference 2)-7), 9), 10) and 16)
Flow cytometry; 10 µg/mL (final concentration)
Immunocytochemistry; Not tested*
Immunohistochemistry; 10 µg/mL

*Reactivity of this clone in this application is not confirmed in our laboratory. However, it is reported that this clone can be used in immunocytochemistry in the reference number 6), 8), 15) and 17).

Detailed procedure is provided in the following **PROTOCOL.**

INTENDED USE:

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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	*Raji	Not tested	Not tested
Reactivity on FCM	+		

* Bromodeoxyuridine incorporated in nuclei in Raji cells

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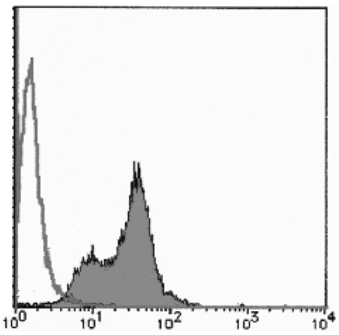

Flow cytometric analysis of BrdU treated Raji cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of MI-11-3 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Add Bromodeoxyuridine (final 10 μM) to the cells.
2) Culture at 37°C in CO_2 incubator for 45 minutes.
3) Add 200 μl of 70% Ethanol to the cell pellet after tapping. Mix well, then fix the cells for 30 minutes at -30°C.
4) Wash the cells 2 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3]. *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
5) Treatment with 1.5 N HCl at room temperature (20~25°C) and incubate at room temperature for 30 minutes to denature the double strand DNA.
6) Wash the cells 1 time with washing buffer.
7) Treatment with 0.1 M $Na_2B_4O_7$ to neutralize the DNA solution.
8) Wash the cells 2 times with washing buffer.
9) Add 10 μL of normal goat serum to the cell pellet after tapping. Mix well, and incubate for 5 minutes at room temperature.
10) Add 30 μL of primary antibody diluted as suggest in **APPLICATIONS** (The concentration of antibody will depend on condition) to cell pellet and gently mix then incubate for 30 minutes at room temperature.
11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
12) Add FITC-conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
13) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
14) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Bromodeoxyuridine incorporated in nuclei in Raji cells)

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