**For Research Use Only.
Not for use in diagnostic procedures.**

MONOCLONAL ANTIBODY

PE labeled Anti-Bromodeoxyuridine

Code No.	Clone	Subclass	Quantity
MI-11-5	2B1	Mouse IgG1	1mL(50 tests)

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: 50 tests in 1mL volume of PBS containing 1% BSA and 0.09% NaN_3.
*Azide may react with copper or lead in plumbing system to form explosive metal azide. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

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

APPLICATION:
Flow cytometry; 20 µL (ready for use)
*Please refer to the data sheet (MBL code no. MI-11-3) for other applications.

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

REFERENCES:
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6) Imai, F., *et al., Development* **133**, 1735-1744 (2006)
7) Itakura, E., *et al., Mol. Biol. Cell* **16**, 5551-5562 (2005)
8) Ito, S., *et al., J. Gen. Virol*. **83**, 2377-2383 (2002)
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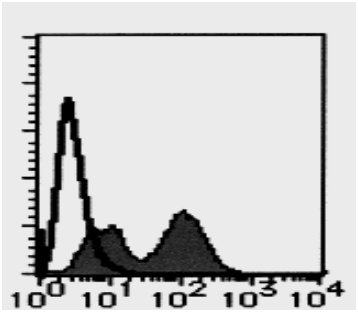
10) Nakajima, T., *et al., PNAS* **95**, 10590-10595 (1998)
11) Gonchoroff, N. J., *et al., J. Immunol. Meth.* **93**, 97-101 (1986)
12) Gonchoroff, N. J., *et al., Cytometry* **6**, 506-512 (1985)
13) Gratzner, H. G., *et al., Cytometry* **1**, 385-389 (1981)

Clone 2B1 is used in reference number 1) - 10).

RELATED PRODUCTS:
MI-11-3 anti-Bromodeoxyuridine (2B1)
M075-3 Mouse IgG1 isotype control (2E12)
M075-5 Mouse IgG1 isotype control-PE (2E12)



Flow cytometric analysis of Bromodeoxyuridine incorporated in nuclei in Raji cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of MI-11-5 to the cells.*

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 minute.
3) Add 200 µl of 70% Ethanol to the cell pellet after tapping. Mix well, then fix the cells for 30 minute at -30°C.
4) Wash the cells 2 times with washing buffer [PBS

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URL https://ruo.mbl.co.jp
e-mail support@mbl.co.jp, TEL 052-238-1904

containing 2% fetal calf serum (FCS) and 0.1% NaN_3].

5) Treatment with 1.5N HCl at room temperature 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 0.1M $Na_2B_4O_7$ 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 (20~25 ^oC).
10) Add 20 μl of primary antibody. Mix well, and incubate for 30 minute 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) 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)