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MBL
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MONOCLONAL ANTIBODY

Anti-PML (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
M041-3	1B9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Acute promyelocytic leukemia (APL) is associated with a t(15;17) translocation that creates the promyelocyte-retinoic acid receptor α (PML-RARα) fusion protein and successfully differentiated by all-*trans*-retinoic acid (ATRA). PML-RARα consists of all amino acid of RARα except the first 59 amino acids and includes its DNA-binding and ligand-binding domains. PML-RARα contains the functional domains of PML which includes the DNA binding and dimerization property. Thus, the functions of PML and/or retinoid X receptor are sequestrated by PML-RARα in a dominant negative manner. In APL cells, the PML-RARα and PML are immunologically localized as microgranules in the nuclei and cytoplasm, whereas in normal cells, PML is immunologically found as a discrete speckled pattern in nuclei. The ATRA treatment of the APL cells triggers a reorganization of PML to generate normal localization. Anti-PML antibody is a strong tool for the detection of the chromosomal translocation t(15;17) on the APL cells and/or determination of the sensitivity of the APL cells to the ATRA differentiation of hematopoietic cells and apoptosis.

SOURCE: This antibody was purified from hybridoma (clone 1B9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant human PML.

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 PML on Immunocytochemistry, Western blotting and Flow cytometry.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	NB-4, HEp-II	Not tested	Not tested
Reactivity on IC	+		

APPLICATIONS:

Western blotting; 10 µg/mL
Immunoprecipitation; Not tested*
*It is reported that this clone 1B9 can be used in Immunoprecipitation in the reference number 2).
Immunohistochemistry; Not tested
Immunocytochemistry; 1 µg/mL
Immunostaining features in the APL cell line NB-4 showed the microgranular pattern. Exposure to 0.1 µM of ATRA for 48 hours restored the normal immunostaining pattern.
Flow cytometry; 10 µg/mL

Detailed procedures are provided in **PROTOCOLS**.

INTENDED USE:

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REFERENCES:

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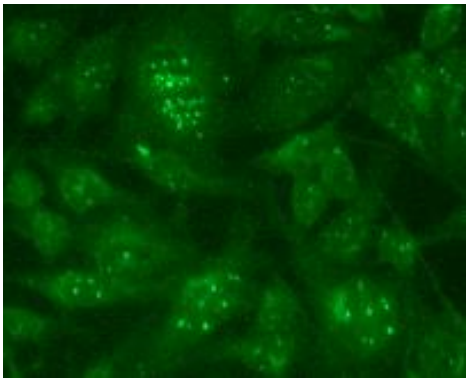
2) Nguyen, L. A., *et al.*, *Blood* **105**, 292-300 (2005) [IP]
3) Mitleman, F., *et al.*, *Oncogene* **6**, 1285-1292 (1988)
4) Yoshida, H., *et al.*, *Cancer Res.* **56**, 2945-2948 (1996)
5) Degos, L., *et al.*, *Blood* **85**, 2643-2653 (1995)
6) Dyck, J. A., *et al.*, *Cell* **76**, 333-343 (1994)
7) Warrell, R. P., *et al.*, *N. Engl. J. Med.* **324**, 1385-1393 (1991)
8) de The, H., *et al.*, *Cell* **66**, 675-684 (1991)
9) Kakizuka, A., *et al.*, *Cell* **66**, 663-674 (1991)
10) de The, H., *et al.*, *Nature* **347**, 558-561 (1990)

Clone 1B9 is used in the reference number 1) and 2).



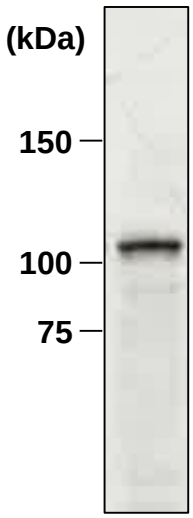
Immunocytochemical detection of PML in acetone fixed HEp-II with M041-3.

PROTOCOLS:

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of cells per one well, then incubate in a CO_2 incubator overnight.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes on ice.
3) Air dry the slides.
4) Add 30 µL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) on to the cells. Incubate for 10 minutes at room temperature.
   *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) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
6) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat another wash once more.
7) Add 30 µL of Alexa Fluor® 488 conjugated anti-mouse IgG (1:500, Thermo Fisher Scientific, code no. A11001) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
8) Wash the slide in a plenty of PBS as in the step 6).
9) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
10) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive controls for Immunocytochemistry; NB-4 and HEp-II)

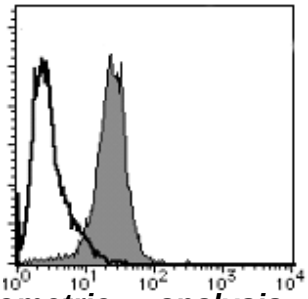


Western blot analysis of PML expression in HeLa using M041-3.

SDS-PAGE & Western blotting

1) Wash the 1 x 10^7 cells 3 times with PBS and resuspend in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 5 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). See the manufacture's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3).
7) Incubate the membrane with 1:10,000 Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL, code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 3).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
10) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; HeLa)



Flow cytometric analysis of PML expression in Jurkat. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of M041-3 to the cells.*

Flow cytometric analysis for floating cells

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

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].

2) Add 200 μL of PBS containing 4% paraformaldehyde to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
3) Wash the cells 3 times with washing buffer.
4) Add 200 μL of 0.1% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 15 minutes at room temperature.
5) Wash the cells 3 times with washing buffer.
6) Add 20 μL of Clear Back (human Fc receptor blocking reagent, MBL, code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 40 μL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) 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.
9) Add 40 μL of Alexa Fluor® 488 conjugated anti-mouse IgG (1:500, Thermo Fisher Scientific, code no. A11001) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
10) 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.
11) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Jurkat)

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