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

MONOCLONAL ANTIBODY

Anti-Mesothelin (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D233-3	B35	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: Mesothelin (MSLN) is a GPI-linked cell surface glycoprotein expressed in the mesothelial lining of the body cavities and in mullerian duct epithelium related cancer cells (ovarian, pancreatic cancer, mesothelioma). Both human and mouse mesothelin bind to ovarian cancer antigen CA125/MUC16 with high affinity, mediating cell attachment *in vitro*, suggesting that mesothelin may facilitate ovarian cancer metastasis.

SOURCE: This antibody was purified from hybridoma (clone B35) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with Wister rat splenocyte immunized with the murine hemangioblast-like cell line LO.

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 mouse mesothelin on Flow cytometry.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Jurkat	LO*	Not tested
Reactivity on FCM	-	+	

*murine hemangioblast-like cell line[ref. 4)]

INTENDED USE:
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REFERENCES:
1) Gubbels, J. A., *et al., Mol. Cancer* **5**, 50-64 (2006)
2) Ho, M., *et al., Clin. Cancer Res.* **11**, 3814-3820 (2005)
3) Rump, A., *et al., J. Biol. Chem.* **279**, 9190-9198 (2004)
4) Nakayama, K., *et al., J. Biol. Chem.* **274**, 24766-24772 (1999)
5) Kojima, T., *et al., J. Biol. Chem.* **270**, 21984-21990 (1995)

Clone B35 is used in reference number 3).

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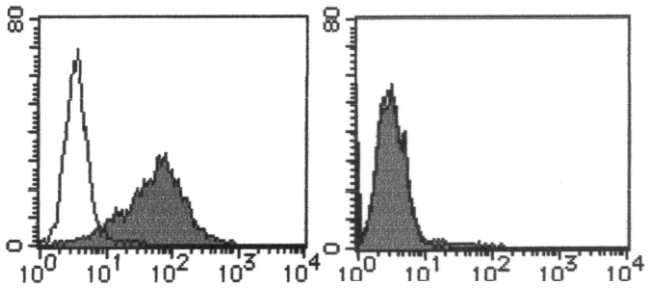

Flow cytometric analysis of mouse Mesothelin expression on LO (left) and Jurkat (right). Open histogram indicates the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D233-3 to the cells.

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) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.05% 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.
2) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 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.

5) Add 30 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.
6) 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.
7) Add FITC conjugated anti-rat IgG antibody diluted with the washing buffer. Mix well and incubate for 15 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LO)