

MONOCLONAL ANTIBODY

Anti-Mesothelin (Mouse) mAb-PE

Code No.	Clone	Subclass	Quantity
D053-5	295D	Rat IgG2a κ	1 mL (50 tests)

BACKGROUND: Mesothelin (MSLN) is a GPI-linked cell surface glycoprotein expressed in the mesothelial lining of the body cavities and in Müllerian 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 295D) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with rat splenocyte immunized with the murine hemangioblast-like cell line LO

FORMULATION: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN₃.

*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.

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

REACTIVITY: This antibody reacts with mouse Mesothelin on Flow cytometry.

APPLICATION:

Flow cytometry; 20 μ L (ready for use)

*Please refer to the data sheet (MBL code no. D053-3) for other applications.

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

SPECIES CROSS REACTIVITY:

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

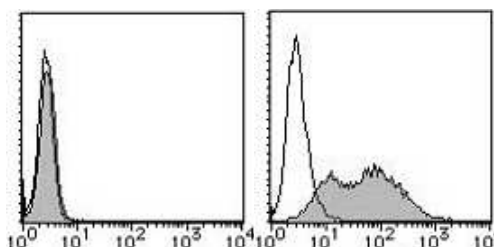
*murine hemangioblast-like cell line^{ref. 4)}

INTENDED USE:

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

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)



Flow cytometric analysis of mouse Mesothelin expression on Jurkat (left) and LO (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of D053-5 to the cells.

PROTOCOL:

Flow cytometric analysis for adherent cells

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

- 1) Detach the cells from culture dish by using cell dissociation buffer (Invitrogen; code no. 13151-014).
- 2) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃].
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- 3) Resuspend the cells with washing buffer (5x10⁶ cells/mL).
- 4) Add 50 μ L 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.
- 5) Add 10 μ L of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN₃ to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
- 6) Add the primary antibody as suggested in the **APPLICATION**. Mix well and incubate for 30 minutes at room temperature.
- 7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.

8) Resuspend the cells with 500 μ L of washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; LO)

RELATED PRODUCTS:

D053-3 Anti-Mesothelin (Mouse) mAb (295D)

D233-3 Anti-Mesothelin (Mouse) mAb (B35)

M081-5 Rat IgG2a (isotype control)-PE (2H3)