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



#### MONOCLONAL ANTIBODY

# Anti-MXRA8 (Human) mAb

Code No. Clone Subclass Quantity Concentration W040-3 2H2G12A Mouse IgG2a  $\kappa$  100  $\mu$ L 1 mg/mL

**BACKGROUND:** Matrix-remodelling associated 8 (MXRA8), also known as limitrin, is a single-pass type I membrane protein, which possesses two immunoglobulin-like domains. MXRA8 is expressed in the spinal cord, brain and various cancer types.

**SOURCE:** This antibody was purified from hybridoma culture supernatant by Protein A affinity column chromatography.

**IMMUNOGEN:** Human MXRA8 expressed Ba/F3 transfectants generated from SST-REX (signal sequence trap by retrovirus-mediated expression screening).

**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 human MXRA8 on Flow cytometry.

# **APPLICATIONS:**

Flow cytometry: 1-10 μg/mL Western blotting; Not tested Immunoprecipitation; Not tested Immunohistochemistry; Not tested Immunocytochemistry; Not tested

Detailed procedure is provided in the following  $\bf PROTOCOL$ .

#### **INTENDED USE:**

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# **Entrez Gene ID:**

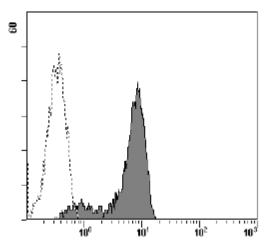
54587 (Human)

# **SPECIES CROSS REACTIVITY:**

| Species           | Human        | Mouse      | Rat        | Hamster    |
|-------------------|--------------|------------|------------|------------|
| Cell              | Transfectant | Not tested | Not tested | Not tested |
| Reactivity on FCM | +            |            |            |            |

## **REFERENCES:**

- 1) Yonezawa, T., et al., Glia 44, 190-204 (2003)
- 2) Kojima, T. and Kitamura, T., Nat. Biotechnol. 17, 487-490 (1999)



Flow cytometric analysis of human MXRA8 expression on Ba/F3 transfectant. Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W040-3 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.

- Wash the cells 3 times with washing buffer [PBS containing 2% fatal calf serum (FCS) and 0.05% NaN<sub>3</sub>].
   \*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 (2.5 x  $10^6$  cells/mL).
- 3) Add 200  $\mu$ L of cell suspension into each tube. And centrifuge at 500 x g for 1 minute at room temperature (20~25°C). Remove supernatant by careful decantation.
- 4) Add 20  $\mu$ L 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.

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- 5) Add 50  $\mu$ L 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 decantation.
- 7) Add 50  $\mu$ L of 1:200 Anti-mouse IgG-PE (Beckman Coulter; code no. IM0855) diluted with 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 decantation.
- 9) Resuspend the cells with 500  $\mu$ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)

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