

**MONOCLONAL ANTIBODY**

# Anti-EphA2 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
W017-3	1H3G4B9	Mouse IgG2a $\kappa$	100 $\mu$ L	1 mg/mL

**BACKGROUND:** Ephrin (EPH) receptor A2 (EPhA2) belongs to the ephrin receptor subfamily of the protein-tyrosine kinase family. EPH and EPH-related receptors have been implicated in mediating developmental events, particularly in the nervous system and in erythropoiesis. Overexpression of EphA2 is observed in a variety of human cancers including breast cancer, prostate cancer, and colorectal cancer. EphA2 also mediates tumor angiogenesis through interaction with its ligand, ephrinA1.

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

**IMMUNOGEN:** Human EphA2 expressed Ba/F3 transfectants generated from SST-REX (signal sequence trap by retrovirus-mediated expression screening).

**FORMULATION:** 100  $\mu$ g IgG in 100  $\mu$ L 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  $-20^{\circ}\text{C}$ .

**REACTIVITY:** This antibody reacts with human EphA2 on Flow cytometry.

**APPLICATIONS:**

- Flow cytometry; 1-10  $\mu$ g/mL
- Western blotting; Not tested
- Immunoprecipitation; Not tested
- Immunohistochemistry; Not tested
- Immunocytochemistry; Not tested

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

**INTENDED USE:**

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

**Entrez Gene ID:**

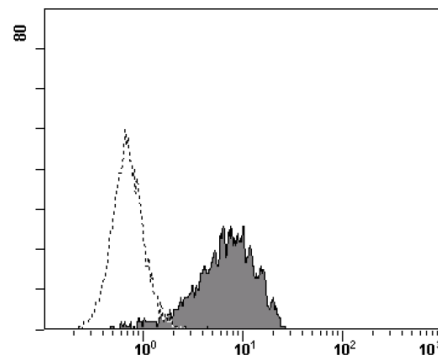
1969 (Human)

**REFERENCES:**

- 1) Hiramoto-Yamaki, N., *et al.*, *J. Cell Biol.* **190**, 461-477 (2010)
- 2) Tanaka, M., *et al.*, *J. Biol. Chem.* **280**, 42375-42382 (2005)
- 3) Miao, H., *et al.*, *Nat. Cell Biol.* **2**, 62-69 (2000)
- 4) Kojima, T and Kitamura, T. *Nat. Biotechnol.* **17**, 487-490 (1999)

**SPECIES CROSS REACTIVITY:**

Species	Human	Mouse	Rat	Hamster
Cells	Transfectant	Not tested	Not tested	Not tested
Reactivity on FCM	+			



**Flow cytometric analysis of human EphA2 expression on Ba/F3 transfectant.** Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W017-3 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.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.05%  $\text{NaN}_3$ ].
- 2) Resuspend the cells with washing buffer ( $2.5 \times 10^6$  cells/mL).
- 3) Add 200  $\mu$ L of cell suspension into each tube. And centrifuge at  $500 \times g$  for 1 minute at room temperature ( $20\sim 25^{\circ}\text{C}$ ). 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.
- 5) Add 50  $\mu$ L of the primary antibody at the concentration as

suggest 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 µL 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 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; transfectant)

#### **RELATED PRODUCTS:**

W005-3	Anti-BTN2A1 (Human) mAb
W008-3	Anti-Carboxypeptidase D (Human) mAb
W011-3	Anti-Dystroglycan (Human) mAb
W017-3	Anti-EphA2 (Human) mAb
W029-3	Anti-IGFBP1 (Human) mAb
W050-3	Anti-RECK (Human) mAb
W086-3	Anti-LYPD3 (C4.4A) (Human) mAb
W089-3	Anti-C11orf24 (Human) mAb
W124-3	Anti-GPR56 (Human) mAb
W125-3	Anti-GPR56 (Human) mAb
W194-3	Anti-FAM171A1 (Human) mAb
W253-3	Anti-Glypican 1 (Human) mAb
W357-3	Anti-CD105 (Endoglin) (Human) mAb
W358-3	Anti-CD300A (Human) mAb
W359-3	Anti-CD300C (Human) mAb
M076-3	Mouse IgG2a (isotype control)
MTG-001	Clear Back (Human Fc receptor blocking reagent)
CY-1092	CycLex <sup>®</sup> EphA2 Kinase Assay/Inhibitor Screening Kit