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

MONOCLONAL ANTIBODY

Anti-ROR2 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
W360-3	6F12A2	Mouse IgG2b κ	100 µL	1 mg/mL

BACKGROUND: Receptor tyrosine kinase-like orphan receptor 2, also known as ROR2, belongs to a family of the receptor tyrosine kinases. While ROR2 is highly expressed during early embryonic development, it has low expression levels in adult tissues. ROR2 has been known to play key roles in developmental morphogenesis, particularly in the formation of cartilage-derived skeleton. Disruption of mouse Ror2 leads to profound skeletal abnormalities. In humans, mutations in *ROR2* are found in brachydactyly type B1, characterized by hypoplasia of the distal and middle phalanges. ROR2 deficiency is also associated with recessive Robinow syndrome, resulting in shortening of limbs, facial dysmorphia, and spinal defects.

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

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

IMMUNOGEN: Human ROR2 expressed Ba/F3 transfectants generated from SST-REX (signal sequence trap by retrovirus-mediated expression screening).

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 ROR2 on Flow cytometry.
*This antibody also can be used for Immunocytochemstry and Western blotting.

APPLICATION-CONFIRMED:
Flow cytometry; 1-10 µg/mL

APPLICATIONS-UNDER EVALUATION:
Western blotting; 1-2 µg/mL
Immunocytochemistry; 2.5-5 µg/mL

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

INTENDED USE:
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Entrez Gene ID:
4920 (Human)

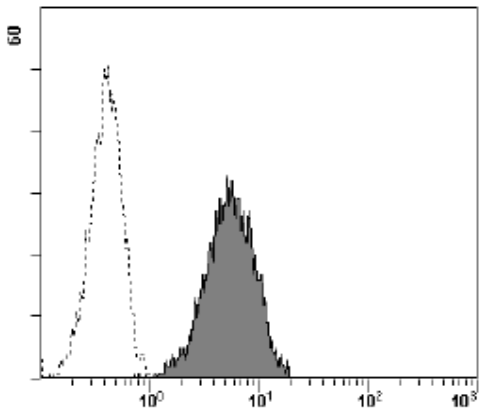
SPECIES CROSS REACTIVITY:

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

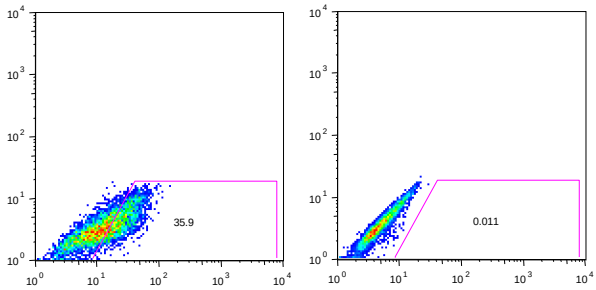
REFERENCES:
1) Liu, Y., *et al*., *Mol. Endocrinol.* **21**, 3050-3061 (2007)
2) van Bolhoven, H., *et al*., *Nat. Genet.* **25**, 423-426 (2000)
3) Afzal, A. R., *et al*., *Nat. Genet.* **25**, 419-422 (2000)
4) Kojima, T. and Kitamura, T., *Nat. Biotechnol.* **17**, 487-490 (1999)

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% fatal calf serum (FCS) and 0.05% NaN_3].
2) Resuspend the cells with washing buffer (2.5×10^6 cells/mL).
3) Add 200 µL 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 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 50 µL 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 Goat F(ab') 2 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)



Flow cytometric analysis of human ROR2 expression on Ba/F3 transfectant. *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W360-3 to the cells.*



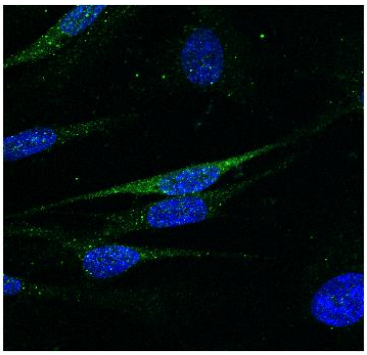
Flow cytometric analysis of ROR2 expression (left) and negative control (right) on human mesenchymal stem cells. *The staining intensity of W360-3 is shown in the horizontal axis.*

Data were kindly provided by Dr. Yumi Matsuzaki. (Department of Life Laboratory of Tumor Biology, Faculty of Medicine, Shimane University)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 mesenchymal stem cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.
4) Wash the glass slide 3 times with PBS.
5) Immerse the slide in PBS containing 0.3% Triton X-100 for 5 minutes at room temperature.
6) Wash the glass slide 3 times with PBS.
7) Immerse the slide in containing 10% goat serum for 30-60 minutes at room temperature.
8) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature (Optimization of antibody concentration or incubation condition are recommended if necessary.)
9) Wash the glass slide 3 times with PBS.
10) Add 100 μL of 1:1,000 Alexa Fluor® 488 Goat Anti-Mouse IgG (Molecular Probes; code no. A11029) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the glass slide 3 times with PBS.
12) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
13) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Human mesenchymal stem cell)



Immunocytochemical detection of ROR2 in human mesenchymal stem cells with W360-3.
Green: Anti-ROR2 (Human) mAb (W360-3)
Blue: DAPI
Fluorescence Microscope: LSM700
Magnification: 630x
Concentration of W360-3: 4 μg/mL

Data was kindly provided by Dr. Yumi Matsuzaki. (Department of Life Laboratory of Tumor Biology, Faculty of Medicine, Shimane University)

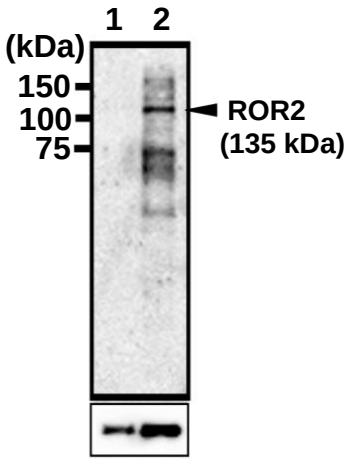
SDS-PAGE & Western Blotting

1) Wash cells (approximately 1 x 10^7 cells) 3 times with PBS and resuspend them in 1 mL of Laemmli's sample buffer.
2) Boil the samples for 3 minutes and centrifuge. Load 20 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% MeOH). See the manufacturer's manual for precise transfer procedure.
4) To reduce nonspecific binding, soak the membrane in 5% 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 PBS (pH 7.2) containing 1% skimmed milk 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 times).
7) Incubate the membrane with 1:10,000 Anti-IgG (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 times).
9) Wipe excess buffer off the membrane, and incubate membrane with an appropriate chemiluminescence reagent for 1 minute.
10) Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
11) Expose the membrane onto an X-ray film in a dark room for 10 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blot analysis for ROR2 expression in untransfected (lane 1) and HA-tagged human ROR2 transfected HEK293T cells (lane 2) using W360-3 (upper) or control β-actin antibody (lower).

Data was kindly provided by Dr. Yumi Matsuzaki. (Department of Life Laboratory of Tumor Biology, Faculty of Medicine, Shimane University)

RELATED PRODUCTS:

W005-3	Anti-BTN2A1 (Human) mAb
W008-3	Anti-Carboxypeptidase D (Human) mAb
W010-3	Anti-CCDC107 (Human) mAb
W011-3	Anti-Dystroglycan (Human) mAb
W017-3	Anti-EphA2 (Human) mAb
W029-3	Anti-IGFBP1 (Human) mAb
W031-3	Anti-IGFBP6 (Human) mAb
W039-3	Anti-MANSC1 (Human) mAb
W041-3	Anti-Neuroplastin (Human) mAb
W046-3	Anti-CD201 (EPCR) (Human) mAb
W049-3	Anti-QSOX1 (Human) mAb
W050-3	Anti-RECK (Human) mAb
W052-3	Anti-Osteopontin (SPP1) (Human) mAb
W072-3	Anti-CD358 (DR6) (Human) mAb
W074-3	Anti-CRELD1 (Human) mAb
W077-3	Anti-GRK5 (Human) mAb
W080-3	Anti-ADAMTS1 (Human) mAb
W086-3	Anti-LYPD3 (C4.4A) (Human) mAb
W089-3	Anti-C11orf24 (Human) mAb
W092-3	Anti-CD321 (F11R/JAM-A) (Human) mAb
W109-3	Anti-TMED2 (Human) mAb
W111-3	Anti-DLL4 (Human) mAb
W117-3	Anti-TINAGL1 (Human) mAb
W124-3	Anti-GPR56 (Human) mAb
W125-3	Anti-GPR56 (Human) mAb
W128-3	Anti-CD318 (CDCP1) (Human) mAb
W147-3	Anti-TYRO3 (Human) mAb
W158-3	Anti-HEXA (Human) mAb
W164-3	Anti-RHBDD3 (Human) mAb
W168-3	Anti-AXL (Human) mAb
W172-3	Anti-CD172a (SIRPα) (Human) mAb
W181-3	Anti-Apolipoprotein D (Human) mAb
W194-3	Anti-FAM171A1 (Human) mAb
W253-3	Anti-Glypican 1 (Human) mAb
W321-3	Anti-FGFRL1 (Human) mAb
W357-3	Anti-CD105 (Endoglin) (Human) mAb
W358-3	Anti-CD300A (Human) mAb
W359-3	Anti-CD300C (Human) mAb
W360-3	Anti-ROR2 (Human) mAb
W361-3	Anti-ROR2 (Human) mAb
W362-3	Anti-FZD5 (Human) mAb
M077-3	Mouse IgG2b (isotype control)
MTG-001	Clear Back (Human Fc receptor blocking reagent)