

MONOCLONAL ANTIBODY

Anti-FZD5 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
W362-3	6F5B9	Mouse IgG2a κ	100 μ L	1 mg/mL

BACKGROUND: Frizzled family receptor 5 (FZD5), also known as C2orf31, is a 7 transmembrane protein that is one of receptors for Wnt signaling proteins. FZD5 is associated with exudative vitreoretinopathy and basal cell carcinoma.

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

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

IMMUNOGEN: Human FZD5 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 FZD5 on Flow cytometry.
*This antibody also can be used for Immunocytochemistry and Western blotting.

APPLICATION-CONFIRMED:
Flow cytometry: 1-10 μ g/mL

APPLICATIONS-UNDER EVALUATION:
Western blotting: 1-2 μ g/mL
Immunocytochemistry: 2.5-5 μ g/mL

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

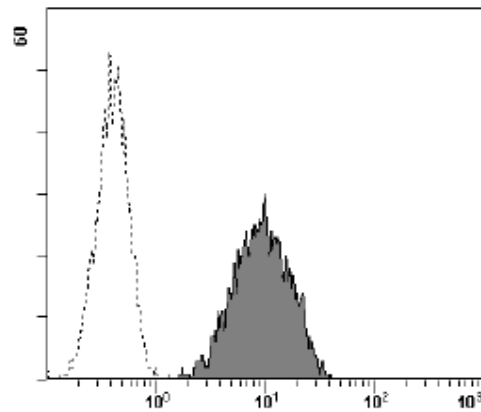
INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

Entrez Gene ID:
7855 (Human)

REFERENCES:
1) Hsieh, J. C., *et al.*, *PNAS* **96**, 3546-3551 (1999)
2) 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 FZD5 expression on Ba/F3 transfectant. Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of W362-3 to the cells.

PROTOCOL:

Flow cytometric analysis for floating cells

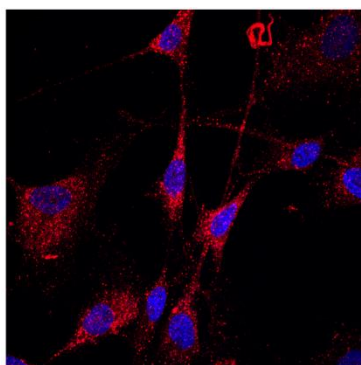
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.05% NaN_3].
- 2) Resuspend the cells with washing buffer (2.5×10^6 cells/mL).
- 3) Add 200 μ 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 μ 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 μ 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 μ L 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 μ L of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; Transfectant)



Immunocytochemical detection of FZD5 in human mesenchymal stem cells with W362-3.

Red: Anti-FZD5 (Human) mAb (W362-3)
Blue: DAPI
Fluorescence Microscope: LSM700
Magnification: 630x
Concentration of W362-3: 4 μ g/mL

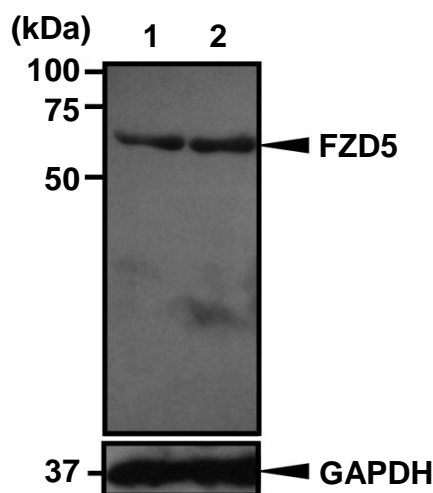
Data was kindly provided by Dr. Yumi Matsuzaki. (Department of Life Laboratory of Tumor Biology, Faculty of Medicine, Shimane University)

Immunocytochemistry

- 1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1×10^4 mesenchymal stem cells for one slide, then incubate in a CO₂ 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 μ L of 1:1,000 Alexa Fluor[®] 555 Goat Anti-Mouse IgG (Molecular Probes; code no. A21422) 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)



Western blot analysis for FZD5 expression in human mesenchymal stem cells using W362-3 (upper) or control GAPDH antibody (lower).

Lane 1: hMSC clone 1
Lane 2: hMSC clone 2

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 1×10^7 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 μ L 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/cm² 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; Human mesenchymal stem cell)

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|---------|---|
| W361-3 | Anti-ROR2 (Human) mAb |
| W362-3 | Anti-FZD5 (Human) mAb |
| M076-3 | Mouse IgG2a (isotype control) |
| MTG-001 | Clear Back (Human Fc receptor blocking reagent) |

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 (SIRP α) (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 |