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

MONOCLONAL ANTIBODY

Anti-GFRα1

Code No.	Clone	Subclass	Quantity	Concentration
M143-3	3H8	Mouse IgG2b	100 µg	1 mg/mL

BACKGROUND: Glial cell line-derived neurotrophic factor (GDNF) family plays a critical role in neurodevelopment and survival of central and peripheral neurons. The biological activity of GDNF family through receptor protein tyrosine kinase Ret and require a ligand specific co-receptor α (GFRα1, binds GDNF, whereas GFRα2 binds NRTN, GFRα3 binds ARTN and GFRα4 binds PSPN). Human GFRα1 is constituted from 465 amino acids and attach to the cell membrane by glycosylphosphatidylinositol (GPI) anchoring manner. It is widely accepted that homodimeric GDNF induce the complex formation of GFRα1 and further the stoichiometry of the signaling complex with $GDNF_2$-$GFR\alpha1_2$-Ret_2, but the mechanism of complex formation remain unclear.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 3H8) was established by fusion of mouse myeloma cell P3U1 with C3H mouse lymphocyte immunized with recombinant human GFRα1 extracellular domain (24-440 aa).

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 GFRα1 on Immunoprecipitation and Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 1 µg/100 µL of cell extract from 2 x 10^5 cells
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 10 µg/mL (final concentration)

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

INTENDED USE:

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SPECIES CROSS REACTIVITY:

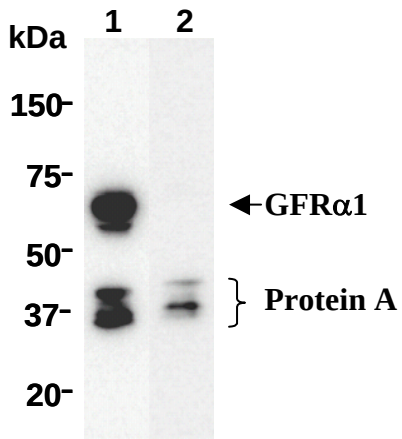
Species	Human		Mouse	Rat
Cells	HCC-1500, MCF-7	BT-20, ZR-75-1	Not Tested	Not Tested
Reactivity on FCM and IP	+	-		

REFERENCES:

1) Leppänen, V-M., *et al.*, *EMBO J.* **23**, 1452-1462 (2004)
2) Wang, L-M., *et al.*, *J. Biol. Chem.* **279**, 109-116 (2004)
3) Eketjäll, S., *et al.*, *EMBO J.* **18**, 5901-5910 (1999)
4) Klein, R. D., *et al.*, *Nature* **387**, 717-724 (1997)
5) Sanicola, M., *et al.*, *PNAS* **94**, 6238-6243 (1997)
6) Jing, S., *et al.*, *Cell* **85**, 1113-1124 (1996)

RELATED PRODUCT:

M142-3 Anti-GFRα1 (4G10, for Western blotting)



Immunoprecipitation of GFRα1 from HCC-1500 with M143-3 (1) or isotype control (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with M142-3.*

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PROTOCOLS:

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of RIPA buffer containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds)
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the **APPLICATIONS** into 100 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
7) 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 manufacture's manual for precise transfer procedure.
8) To reduce nonspecific binding, soak the membrane in 4% Block Ace for 1 hour at room temperature, or overnight at 4°C.
9) Incubate the membrane with 1 µg/mL of Anti-GFRα1 (Clone 4G10, MBL; code no. M142-3) as primary antibody diluted with 4% Block Ace for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with the 1:2,000 HRP-conjugated anti-mouse IgG (eBioscience; code no. 18-8877) diluted with 1% BSA (in PBS, pH 7.2) for 1 hour at room temperature.
12) Wash the membrane with PBS-T (5 minutes x 6 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 2 minutes. Develop the film as usual. The condition for exposure and development may vary.

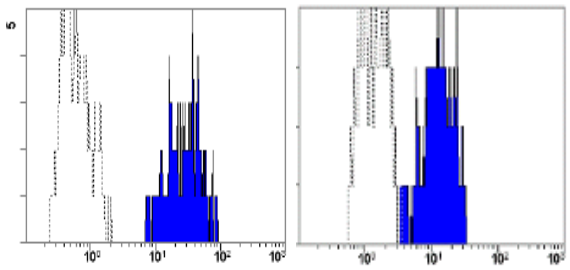
(Positive controls for Immunoprecipitation: HCC-1500, MCF-7)

Flow cytometric analysis for adherent cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps after 2).

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 0.1% NaN_3].
3) Resuspend the cells with washing buffer ($5x10^6$ cells/mL).
4) Add 100 µL 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 20 µL of the primary antibody at the concentration of 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 aspiration.
7) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with the washing buffer. Mix well and incubate for 15 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 aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive controls for Flow cytometry; HCC-1500, MCF-7)



Flow cytometric analysis of GFRα1 expression on HCC-1500 (left) and MCF-7 (right). *Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of M143-3 to the cells.*