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Product datasheet for AM26645AF-N

GDNF Receptor alpha 1 (GFRA1) (alpha) (24-440) Mouse Monoclonal Antibody [Clone ID: 3H8]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3H8
Applications:	FC, IP
Recommended Dilution:	Immunoprecipitation: 1 µg/100 µl of cell extract from 2 x 10e5 cells. Flow cytometry: 10 µg/ml (final concentration). For details see protocols below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Recombinant human GFRα1 extracellular domain (24-440 aa)
Specificity:	This antibody detects GFRα1. reacts with HCC-1500, MCF-7, but **NOT** with BT-20, ZR-75-1.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	GDNF family receptor alpha 1
Database Link:	Entrez Gene 2674 Human P56159

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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 a (GFRa1, binds GDNF, whereas GFRa2 binds NRTN, GFRa3 binds ARTN and GFRa4 binds PSPN). Human GFRa1 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 GFRa1 and further the stoichiometry of the signaling complex with GDNF2-GFRa12-Ret2, but the mechanism of complex formation remain unclear.

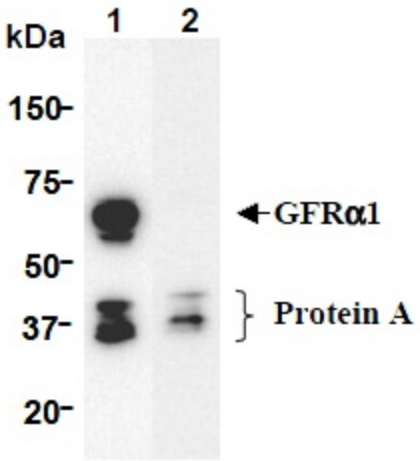
Synonyms: GFR-alpha-1, GDNF receptor alpha, RET ligand 1, GDNFRA, RETL1, TRNR1

Note: This product was originally produced by MBL International.

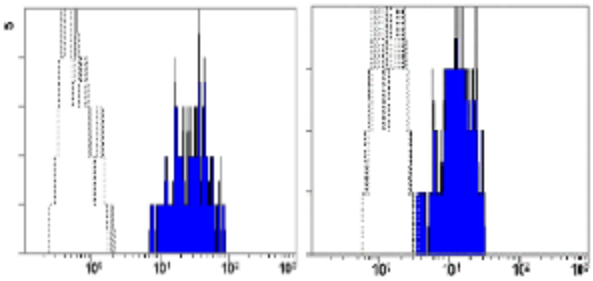
Protocol:
Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volume of RIPA buffer containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds)
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and 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 4oC. 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 4oC.
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/cm2 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 4oC.
9) Incubate the membrane with 1 µg/mL of Anti-GFRa1 (Clone 4G10) 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 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% NaN3].
3) Resuspend the cells with washing buffer (5x10e6 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 (20~25oC). 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 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)

Product images:



Immunoprecipitation of GFRα1 from HCC-1500 with AM26645AF-N (1) or isotype control (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26645AF-N.



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 AM26645AF-N to the cells.