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

POLYCLONAL ANTIBODY

Anti-Go α (GTP Binding Protein Go α Subunit)

Code No	Quantity	Form
551	100 µL	Affinity Purified

BACKGROUND: The heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) mediate signaling from transmembrane cell surface receptors to a variety of intracellular effectors. G-proteins are composed of a 36-52 kDa α-subunit, a 35-36 kDa β-subunit, and an 8-10 kDa γ-subunit. The α subunits bind and hydrolyze GTP and are involved in regulation of intracellular effectors. In mammals, G α proteins are encoded by at least 16 genes.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with bovine Go α.

FORMULATION: 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 Go α (39 kDa) on Western blotting, Immunoprecipitation and Immunohistochemistry.

APPLICATIONS:

Western blotting; 1:2,000 for chemiluminescence detection system
Immunoprecipitation; 5 µL/200 µL of cell extract from 5 x 10^6 cells or tissue extract.
Immunohistochemistry (paraffin section); 1:400 - 1:1,000
Immunohistochemistry (frozen section); 1:500*
*It is reported that this antibody can be used in this applications in the reference number 1).
Immunocytochemistry; Not tested.
Flow cytometry; Not tested

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

PECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells and Tissues	SK-N-SH	brain	brain, PC12
Reactivity on WB	+	+	+

REFERENCES:

1) Prince, J. E. A. *et al., J. Neurosci.* **29**, 14211-14222 (2009)
2) Takigami, S., *et al., Chem. Senses* **29**, 301-310 (2004)
3) Matsuoka, M., *et al., Chem. Senses* **26**, 161-166 (2001)
4) Takigami, S., *et al., Chem. Senses* **25**, 387-393 (2000)
5) Krsmanovic, L. Z., *et al., Endocrinology* **139**, 4037-4043 (1998)
6) Kato, K., *et al., Cancer Res.* **47**, 5800-5805 (1987)
7) Asano, T., *et al., J. Neurochem.* **48**, 1617-1623 (1987)
8) Worley, P.F., *et al., PNAS* **83**, 4561-4565 (1986)

This antibody is used in reference number 1) - 6).

INTENDED USE:

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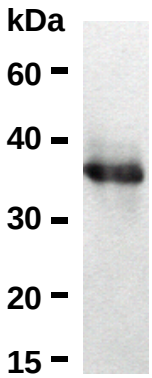


Western blot analysis of Goα expression in rat brain using 551.

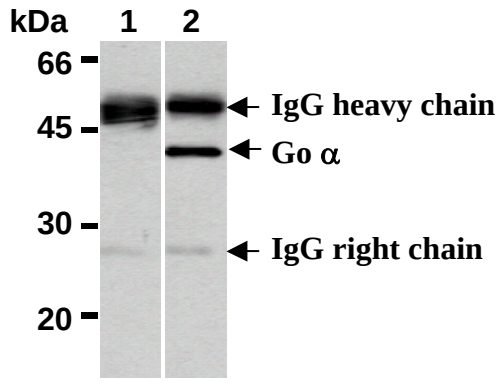
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Homogenize the tissue in cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM PMSF, 1 mM EDTA) containing appropriate protease inhibitors. Sonicate it briefly (up to 10 seconds) at 4°C.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) 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.

6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; rat brain)



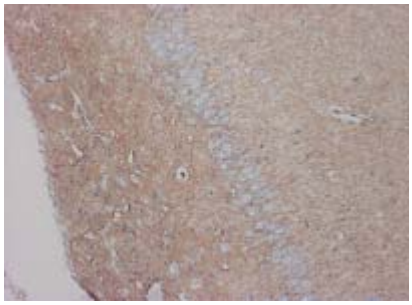
Immunoprecipitation of Goα from rat brain with normal rabbit IgG (1) or 551 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with 551.*

Immunoprecipitation

1) Homogenize the tissue in cold Lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% NP-40, 1 mM PMSF, 1 mM EDTA) containing appropriate protease inhibitors. Sonicate it briefly (up to 10 seconds) at 4°C.
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 suggested in the **APPLICATIONS** into 200 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 beads 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. Use 10 µL/lane for the SDS-PAGE analysis.

(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; rat brain)



Immunohistochemical detection of Goα on rat brain paraffin embedded section with 551.

Immunohistochemical staining for paraffin-embedded section; SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; rat brain)