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

RiboCluster Profiler™ RBP Antibody

Anti-G3BP1 pAb

CODE No. RN048PW

CLONALITY Polyclonal
ISOTYPE Rabbit Ig, affinity purified
QUANTITY 100 µL

SOURCE Purified Ig from rabbit serum
FORMULATION 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.

APPLICATIONS-CONFIRMED:

Western blotting	1:1,000
Immunoprecipitation	5 µL/500 µL of cell extract from 5 x 10^6 cells

APPLICATION-REPORTED:

Immunocytochemistry	Reference 1) and 2)

SPECIES CROSS REACTIVITY on WB:

Species	Human	Mouse	Rat	Hamster
Cells	293T, HeLa, Jurkat	NIH/3T3	Rat1	CHO
Reactivity	+	–	–	–

Entrez Gene ID: 10146 (Human)

REFERENCES

1) Markmiller, S., *et al.*, *Cell* **172**, 590-604 (2018) [IC]
2) Sundararaman, B., *et al.*, *Mol. Cell* **61**, 903-913 (2016) [IC]

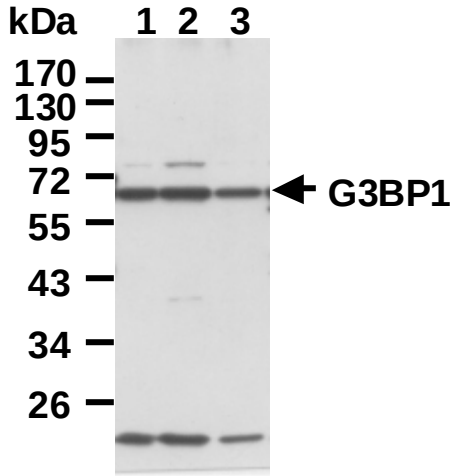
For more information, please visit our web site at https://ruo.mbl.co.jp/.

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Wash 1 x 10^7 cells 3 times with PBS and suspend them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 20 sec.).
2) Boil the samples for 2 min. and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% Methanol). See the manufacture'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 hr. at room temperature, or overnight at 4°C.
5) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 min. x 3 times).
7) Incubate the membrane with 1:5,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
8) Wash the membrane with PBS-T (10 min. x 3 times).
9) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
10) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; 293T, HeLa and Jurkat)



Western blotting analysis of G3BP1

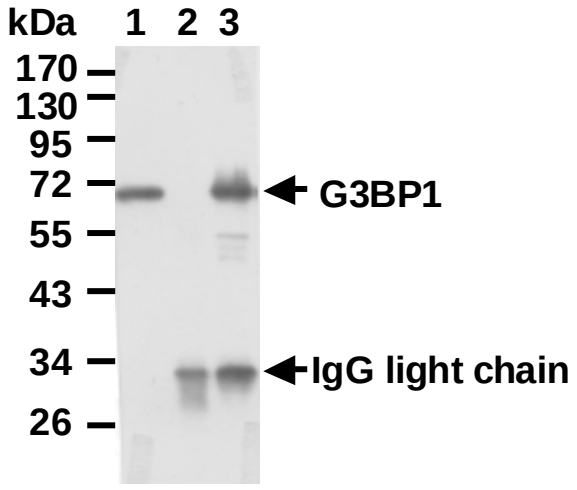
Lane 1: 293T
Lane 2: HeLa
Lane 3: Jurkat

Immunoblotted with Anti-G3BP1 pAb (RN048PW)

Immunoprecipitation

1) Wash 1 x 10^7 cells 2 times with PBS and resuspend them with 1 mL of ice-cold Lysis Buffer (+) (MBL; code no. RN1001) containing appropriate protease inhibitors and DTT. Vortex thoroughly, then incubate on ice for 10 min.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add 40 µL of 50% protein A agarose beads slurry resuspended in ice-cold Wash Buffer (+) (MBL; code no. RN1001) containing DTT at the appropriate concentration into the supernatant. Incubate it at 4°C with rotating for 1 hr.
4) Centrifuge the tube at 2,000 x g for 1 min. at 4°C and transfer the supernatant to another tube (precleared sample).
5) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 1 mL of ice-cold Wash Buffer (+) with Normal Rabbit IgG (RIP-Assay Kit) or Anti-G3BP1 pAb (RN048PW) at the amount of suggested in the **APPLICATIONS,** and then add into each tube. Incubate with gentle agitation for 3 hr. at 4°C.
6) Centrifuge the tube at 2,000 x g for 10 seconds and carefully discard the supernatant using a pipettor without disturbing the beads.
7) Resuspend the beads with ice-cold Lysis Buffer (+).
8) Centrifuge the tube at 2,000 x g for 10 seconds and carefully discard the supernatant using a pipettor without disturbing the beads.
9) Add 500 µL of cell lysate (precleared sample of step 4), then incubate with gentle agitation for 3 hr. at 4°C.
10) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
11) Resuspend the beads with 1 mL of ice-cold Wash Buffer (+).
12) Centrifuge the tube at 2,000 x g for 10 seconds and discard the supernatant.
13) Repeat steps 11)-12) 4 times.
14) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 min., and centrifuge for 5 min. Load 20 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
15) 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% Methanol). See the manufacture's manual for precise transfer procedure.
16) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature, or overnight at 4°C.
17) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
18) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (10 min. x 3 times).
19) Incubate the membrane with the 1:1,000 Rabbit TrueBlot® anti-Rabbit IgG-HRP (eBioscience; code no. 18-8816-33) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
20) Wash the membrane with PBS-T (10 min. x 3 times).
21) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
22) Expose to an X-ray film in a dark room for 3 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; 293T)



Immunoprecipitation of G3BP1 from 293T

Lane 1: Input
Lane 2: Normal Rabbit IgG
Lane 3: Anti-G3BP1 pAb (RN048PW)

Immunoblotted with RN048PW