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

Smart-IP Series

Anti-GFP (Green Fluorescent Protein) mAb -Magnetic Agarose

CODE No.	D153-10
CLONALITY	Monoclonal
CLONE	RQ2
ISOTYPE	Rat IgG2a κ
QUANTITY	20 tests (Slurry: 400 µL)
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	GFP purified from GFP expressed 293T
REACTIVITY	This antibody reacts with GFP, EBFP, ECFP, EGFP, Venus and Sapphire.
FORMULATION	100 µg of antibody is covalently coupled to 400 µL of magnetic agarose gel slurry suspended in PBS/0.1% ProClin 150.
STORAGE	This gel slurry is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

Immunoprecipitation	20 µL of slurry/ 400 µL of cell extract from 2 x 10^6 cells

REFERENCES

1) Yeom, J., *et al., Mol. Cell* **66**, 234-246.e5 (2017) [IP]
2) Yasuda, S., *et al., Mol. Plant.* **10**, 605-618 (2017) [Co-IP]
3) Oh, E. T., *et al., Nat. Commun.* **7**, 13593 (2016) [IP]
4) Cai, L., *et al., J. Biol. Chem*. **286**, 35915-35921 (2011)
5) Sato, Y., *et al., J. Biol. Chem*. **284**, 11873-11881 (2009)
6) Sakurai, T., *et al., J. Cell Biol*. **183**, 339-352 (2008)

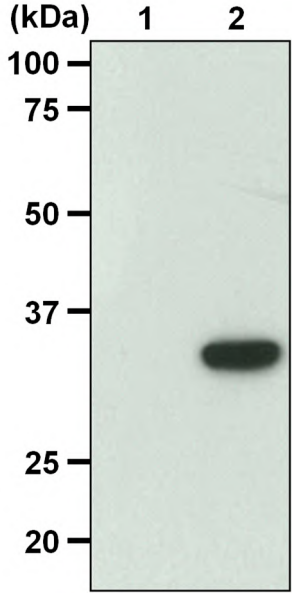
For more information, please visit our website at **https://ruo.mbl.co.jp/**.

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
A JSR Life Sciences Company
URL https://ruo.mbl.co.jp e-mail support@mbl.co.jp

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

Immunoprecipitation

1) Wash 2 x 10^6 cells 3 times with PBS and suspends them in 400 µL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40], then sonicate briefly (up to 20 sec.).
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Add magnetic beads as suggested in the **APPLICATION** into 400 µL of the cell lysate. Mix well and incubate with gentle agitation for 30 min. at 4°C.
4) Place the tube on the magnetic rack (MBL, code no. 3190) for a few seconds.
5) Remove the supernatant.
6) Add 1 mL of cold Lysis buffer and resuspend the magnetic beads.
7) Place the tube on the magnetic rack for a few seconds.
8) Remove the supernatant.
9) Repeat Steps 6)-8) 4 times.
10) Resuspend the magnetic beads in 50 µL of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
11) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.
12) 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 manufacturer's manual for precise transfer procedure.
13) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
14) Incubate the membrane with 1:5,000 of Anti-GFP pAb-HRP-DirecT (MBL, code no. 598-7) diluted with 1% skimmed milk (in PBS, pH 7.2) PBS for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
15) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3).
16) 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.
17) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual settings. The condition for exposure and development may vary.



Immunoprecipitation of GFP-fusion protein
Lane 1: Parental cell (293T)
Lane 2: GFP-fusion protein/293T

Immunoblotted with Anti-GFP pAb-HRP-DirecT (MBL, code no. 598-7)