

Smart-IP Series

# Anti-GFP (Green Fluorescent Protein) mAb -Magnetic Beads

**CODE No.** D153-11

**CLONALITY** Monoclonal  
**CLONE** RQ2  
**ISOTYPE** Rat IgG2a  $\kappa$   
**QUANTITY** 20 tests (Slurry: 1 mL)

**SOURCE** Purified IgG from hybridoma supernatant  
**IMMUNOGEN** GFP purified from GFP expressed 293T cells  
**REACTIVITY** This antibody reacts with EBFP, ECFP, EGFP, Venus and Sapphire as well as GFP.  
**FORMULATION** 10 mg magnetic beads in 1 mL PBS/0.1% BSA/0.09% NaN<sub>3</sub>

\*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

**STORAGE** This beads suspension is stable for one year from the date of purchase when stored at 4°C.

## APPLICATION-CONFIRMED

Immunoprecipitation 50  $\mu$ L of beads slurry/sample

\*The purification capacity of Anti-GFP (Green Fluorescent Protein) mAb-Magnetic Beads varies depending upon the characteristics of a GFP fusion protein. For example, 50  $\mu$ L of beads slurry bounds 4.2  $\mu$ g of a GFP fusion protein (32 kDa).

## APPLICATIONS-REPORTED

Co-Immunoprecipitation Reference 3)

RNP Immunoprecipitation (RIP) Reference 6)

## REFERENCES

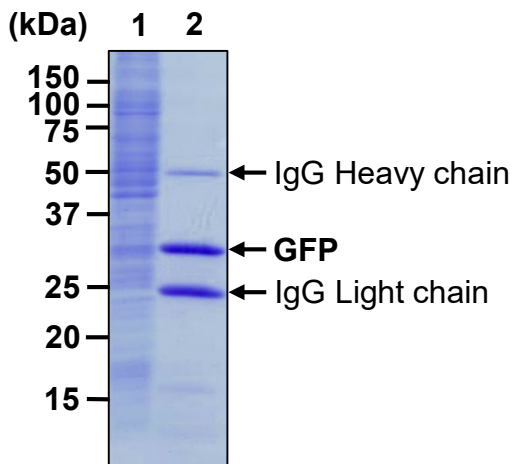
- 1) Zhang, D., *et al.*, *Rice*. **11**, 45 (2018) [IP]
- 2) Jessen, T. N. and Jessen, J. R., *Exp. Cell Res.* **361**, 265-276 (2017) [IP]
- 3) Aoyama, S., *et al.*, *Biochem. Biophys. Res. Commun.* **491**, 33-39 (2017) [IP]
- 4) Chen, C. C., *et al.*, *J. Biol. Chem.* **292**, 12560-12576 (2017) [Co-IP]
- 5) Amara, C. S., *et al.*, *Front. Cell Dev. Biol.* **5**, 20 (2017) [IP]
- 6) Sommer, G., *et al.*, *PLoS One* **12**, e0173246 (2017) [RIP]
- 7) Wang, Y., *et al.*, *Plant Physiol.* **173**, 1235-1246 (2016) [IP]

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

### **Immunoprecipitation**

- 1) Wash  $1 \times 10^7$  cells 3 times with PBS and suspend with 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40] containing appropriate protease inhibitors.
- 2) Centrifuge the tube at  $12,000 \times g$  for 5 min. at  $4^\circ\text{C}$  and transfer the supernatant to another tube.
- 3) Add magnetic beads as suggested in the **APPLICATION** and purified GFP protein into 300  $\mu\text{L}$  of the supernatant prepared in step 2). Mix well and incubate with gentle agitation for 1 hr. at  $4^\circ\text{C}$ .
- 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 Wash buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] 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) 3 times.
- 10) Resuspend the magnetic beads in 20  $\mu\text{L}$  of Laemmli's sample buffer, boil for 2 min., and place the tube on the magnetic rack for a few seconds.
- 11) Load 20  $\mu\text{L}$  of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 12) Visualize the protein bands by CBB staining.



### ***Immunoprecipitation of GFP protein***

Sample: 293T cell lysate from  $3 \times 10^6$  cells + GFP protein 10  $\mu\text{g}$   
Lane 1: Input (5  $\mu\text{L}/\text{lane}$ )  
Lane 2: Post-IP beads of Anti-GFP mAb (D153-11)