

Smart-IP Series

# Anti-RFP mAb-Magnetic Beads

**CODE No.** M165-11

**CLONALITY** Monoclonal  
**CLONE** 3G5  
**ISOTYPE** Mouse IgG1  $\kappa$   
**QUANTITY** 20 tests (Slurry: 1 mL)

**SOURCE** Purified IgG from hybridoma supernatant  
**IMMUNOGEN** RFP  
**REACTIVITY** This antibody reacts with DsRed, mRFP1, mCherry, mOrange and mPlum.  
**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-RFP mAb-Magnetic Beads varies depending upon the characteristics of an RFP fused protein. For example, 50  $\mu$ L of beads slurry bounds 1.5  $\mu$ g of DsRed protein.

**REFERENCES**

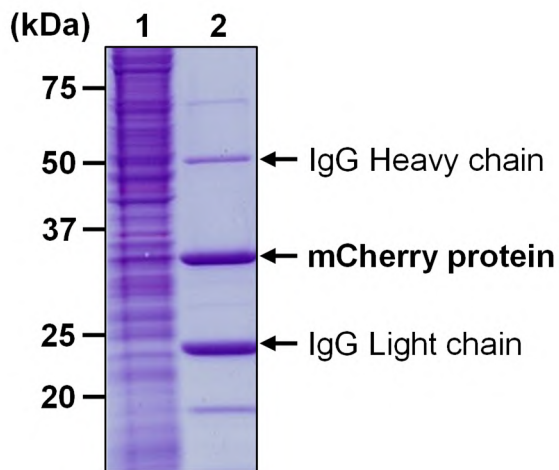
- 1) Södersten, E., *et al.*, *Nat. Commun.* **9**, 1226 (2018) [IP]
- 2) Hao le, T., *et al.*, *J. Neurosci.* **37**, 11559-11571 (2017) [IP]
- 3) Furlow, P. W., *et al.*, *Nat. Cell Biol.* **17**, 943-952 (2015) [IP]

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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 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 300  $\mu$ L of the supernatant prepared in step 2). Mix well and incubate with gentle agitation for 1 hr. at 4°C.
- 4) Place the tube on the magnetic rack (MBL; code no. 3190) for a few seconds.
- 5) Remove the supernatant.
- 6) Wash the beads 4 times with 1 mL of cold Wash buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] (place the tube on the magnetic rack for a few seconds).
- 7) Resuspend the magnetic beads in 20  $\mu$ L of Laemmli's sample buffer, boil for 2 min., and place the tube on the magnetic rack for a few seconds.
- 8) Load 20  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 9) Visualize the protein bands by CBB staining.



#### ***Immunoprecipitation of RFP (mCherry)***

Sample: 293T cell lysate from  $3 \times 10^6$  cells + mCherry protein\* 10  $\mu$ g

Lane 1: Input (10  $\mu$ L/lane)

Lane 2: Post-IP beads of Anti-RFP mAb (M165-11)

\*Sample was provided by RIKEN.