

*Smart-IP Series*

# Anti-DDDDK-tag mAb-Magnetic Beads

<b>CODE No.</b>	M185-11R
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	FLA-1GS
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	20 tests (Slurry: 1 mL)
<b>SOURCE</b>	Purified IgG from CHO cell culture supernatant
<b>IMMUNOGEN</b>	KLH conjugated DYKDDDDK peptide
<b>REACTIVITY</b>	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged (DYKDDDDK) proteins.
<b>FORMULATION</b>	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.
<b>STORAGE</b>	This beads suspension is stable for one year from the date of purchase when stored at 4°C. If bead agglomeration is observed, please disperse the agglomerations by careful pipetting. *In particular, please check the inner wall of the vial and cap.

## APPLICATION-CONFIRMED

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

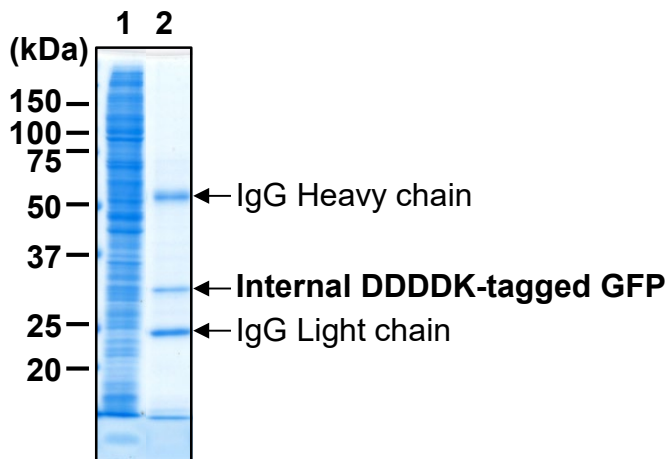
\*The purification capacity of Anti-DDDDK-tag mAb-Magnetic Beads varies depending upon the characteristics of a DDDDK-tagged protein. For example, 50  $\mu$ L of beads slurry bounds  $\geq 0.5$   $\mu$ g of a DDDDK-tagged protein (32 kDa).

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

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, 0.05% 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** 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 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) 3 times.
- 10) Resuspend the magnetic beads in 20  $\mu\text{L}$  of Laemmli's sample buffer, boil for 3 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 DDDDK-tagged protein***

Sample: Internal DDDDK-tagged GFP/293T

Lane 1: Input (5  $\mu\text{L}$ /lane)

Lane 2: Post-IP beads of Anti-DDDDK-tag mAb (M185-11R)