

Smart-IP Series

Anti-DDDDK-tag mAb-Magnetic Agarose

CODE No.	M185-10R
CLONALITY	Monoclonal
CLONE	FLA-1GS
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 tests (Slurry: 2 mL)
SOURCE	Purified IgG from CHO cell culture supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, DYKDDDDK (DDDDK-tag)
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged proteins.
FORMULATION	2 mg of antibody is covalently coupled to 2 mL of magnetic agarose gel slurry suspended in PBS/0.09% NaN ₃ *
	*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 gel slurry is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

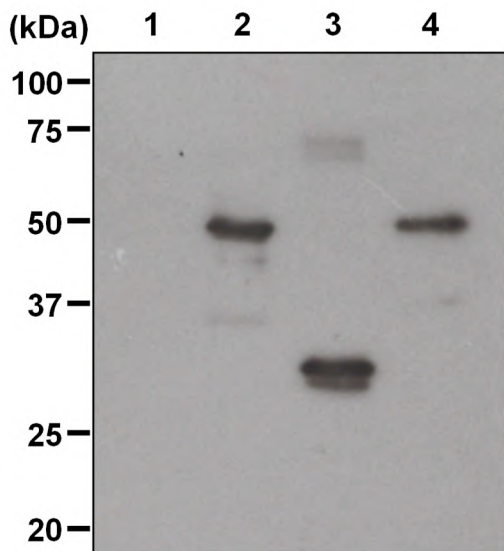
Immunoprecipitation 20 μ L of slurry/400 μ L of cell extract from 1 x 10⁶ cells

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 1×10^6 cells 3 times with PBS and suspend them in 400 μ L of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40], then sonicate briefly (up to 10 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 μ L 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) 3 times.
- 10) Resuspend the magnetic beads in 50 μ L of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
- 11) Load 5 μ L 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/cm² 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) for 1 hr. at room temperature.
- 14) Incubate the membrane with 1:10,000 of Anti-DDDDK-tag mAb-HRP-Direct (MBL, code no. M185-7) 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 DDDDK-tagged protein

Lane 1: 293T cell lysate

Lane 2: Met-N-terminal DDDDK-tagged protein X (1 μ g) in 293T lysate

Lane 3: Internal DDDDK-tagged GFP (1 μ g) in 293T lysate

Lane 4: C-terminal DDDDK-tagged protein X (1 μ g) in 293T lysate

Immunoblotted with Anti-DDDDK-tag mAb-HRP-Direct (MBL, code no. M185-7)