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

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/0.1% ProClin 150
STORAGE	This gel slurry is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

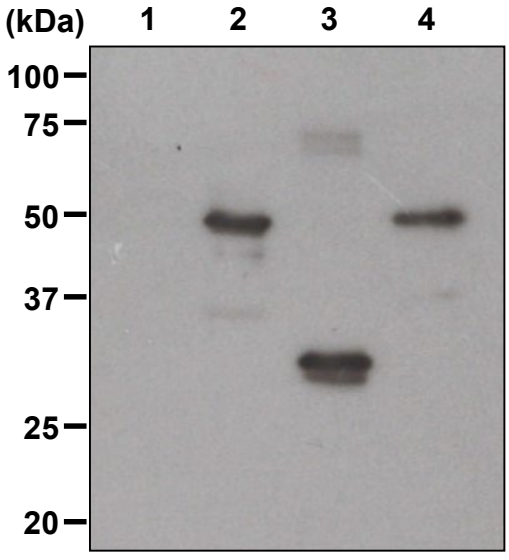
Immunoprecipitation	20 µL of slurry/400 µL of cell extract from 1 x 10^6 cells

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 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 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 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) 3 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 5 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) 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 µg) in 293T lysate
Lane 3: Internal DDDDK-tagged GFP (1 µg) in 293T lysate
Lane 4: C-terminal DDDDK-tagged protein X (1 µg) in 293T lysate

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