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

Smart-IP Series

Anti-DDDDK-tag mAb-Magnetic Agarose

**CODE No.**  
M185-10

**CLONALITY**  
Monoclonal

**CLONE**  
FLA-1

**ISOTYPE**  
Mouse IgG2a κ

**QUANTITY**  
100 tests (Gel: 1 mL)

**SOURCE**  
Purified IgG from hybridoma 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 1 mL of magnetic agarose gel and provided as 2 mL 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  
10 μL of gel/400 μL of cell extract from 1 x 10⁶ cells

**REFERENCES**


For more information, please visit our web site [http://ruo.mbl.co.jp/](http://ruo.mbl.co.jp/)
RELATED PRODUCTS

**Smart-IP series**

- 3190  Magnetic Rack
- D153-10  Anti-GFP mAb-Magnetic Agarose (RQ2)
- M165-10  Anti-RFP mAb-Magnetic Agarose (3G5)
- M047-10  Anti-Myc-tag mAb-Magnetic Agarose (PL14)
- M185-10  Anti-DDDDK-tag mAb-Magnetic Agarose (PLA-1)
- D291-10  Anti-His-tag mAb-Magnetic Agarose (O Geographic)
- M132-10  Anti-HA-tag mAb-Magnetic Agarose (5D8)
- M180-10  Anti-HA-tag mAb-Magnetic Agarose (TANA2)
- M167-10  Anti-V5-tag mAb-Magnetic Agarose (1H6)
- M198-10  Anti-E-tag mAb-Magnetic Agarose (21D11)
- D153-11  Anti-GFP mAb-Magnetic Beads (RQ2)
- M165-11  Anti-RFP mAb-Magnetic Beads (3G5)
- M047-11  Anti-Myc-tag mAb-Magnetic Beads (PL14)
- M185-11  Anti-DDDDK-tag mAb-Magnetic Beads (PLA-1)
- D291-11  Anti-His-tag mAb-Magnetic Beads (O Geographic)
- M132-11  Anti-HA-tag mAb-Magnetic Beads (5D8)
- M180-11  Anti-HA-tag mAb-Magnetic Beads (TANA2)
- M167-11  Anti-V5-tag mAb-Magnetic Beads (1H6)
- M215-11  Anti-V5-tag mAb-Magnetic Beads (OZA3)
- M198-9  Anti-E-tag mAb-Magnetic beads (21D11)
- D058-11  Anti-Multi Ubiquitin mAb-Magnetic Beads (FK2)

**Antibodies**

- D153-3  Anti-GFP mAb (RQ2)
- D153-8  Anti-GFP mAb-Agarose (RQ2)
- M048-3  Anti-GFP mAb (1E4)
- 598  Anti-GFP pAb
- 598-7  Anti-GFP pAb-HRP-DirecT
- M165-3  Anti-RFP mAb (3G5)
- M165-8  Anti-RFP mAb-Agarose (3G5)
- M204-3  Anti-RFP mAb (1G9)
- M208-3  Anti-RFP mAb Cocktail (1G9, 3G5 (mixed))
- M155-3  Anti-RFP mAb (8D6)
- PM005  Anti-RFP pAb
- PM005-7  Anti-RFP pAb-HRP-DirecT
- M192-3  Anti-Myc-tag mAb (My3) (200 µL)
- M047-3  Anti-Myc-tag mAb (PL14)
- M047-7  Anti-Myc-tag mAb-HRP-DirecT (PL14)
- M047-8  Anti-Myc-tag mAb-Agarose (PL14)
- 562  Anti-Myc-tag pAb (0.1 mL)
- M185-3S  Anti-DDDDK-tag mAb (FLA-1) (50 µL)
- M185-3L  Anti-DDDDK-tag mAb (FLA-1) (1 mL)
- M185-3LL  Anti-DDDDK-tag mAb (FLA-1) (5 mL)
- M185-6  Anti-DDDDK-tag mAb-Biotin (FLA-1)
- M185-7  Anti-DDDDK-tag mAb-HRP-DirecT (FLA-1)
- M185-A48  Anti-DDDDK-tag mAb-Alexa Fluor® 488 (FLA-1)
- M185-A59  Anti-DDDDK-tag mAb-Alexa Fluor® 594 (FLA-1)
- M185-A64  Anti-DDDDK-tag mAb-Alexa Fluor® 647 (FLA-1)
- PM020  Anti-DDDDK-tag pAb
- PM020-7  Anti-DDDDK-tag pAb HRP-DirecT
- PM020-8  Anti-DDDDK-tag pAb-Agarose
- M180-3  Anti-HA-tag mAb (TANA2) (200 µL)
- M180-7  Anti-HA-tag mAb-HRP-DirecT (TANA2)
- 561  Anti-HA-tag pAb (0.1 mL)
- 561-7  Anti-HA-tag pAb HRP-DirecT
- 561-8  Anti-HA-tag pAb-Agarose
- M132-3  Anti-HA-tag mAb (5D8)
- D291-3  Anti-His-tag mAb (O Geographic) (200 µL)
- D291-6  Anti-His-tag mAb-Biotin (O Geographic)
- D291-7  Anti-His-tag mAb HRP-DirecT (O Geographic)
- D291-8  Anti-His-tag mAb-Agarose (O Geographic)
- M089-3  Anti-His-tag mAb (6C4)
- M136-3  Anti-His-tag mAb (2D8)
- PM032  Anti-His-tag pAb
- PM032-8  Anti-His-tag pAb-Agarose
- M167-3  Anti-V5-tag mAb (1H6)
- M215-3  Anti-V5-tag mAb (OZA3)
- M215-7  Anti-V5-tag mAb-HRP-DirecT (OZA3)
- PM003  Anti-V5-tag pAb
- PM003-7  Anti-V5-tag pAb-HRP-DirecT
- PM003-8  Anti-V5-tag pAb-Agarose
- PM021  Anti-S-tag pAb
- PM021-8  Anti-S-tag pAb-Agarose
- PM022  Anti-T7-tag pAb
- PM022-8  Anti-T7-tag pAb-Agarose
- 563  Anti-VSV-G-tag pAb
- 563-8  Anti-VSV-G-tag pAb-Agarose
- M209-3  Anti-GST-tag mAb (GTS)
- M071-3  Anti-GST-tag mAb (3B2)
- PM013  Anti-GST-tag pAb
- PM013-7  Anti-GST-tag pAb-HRP-DirecT
- PM070  Anti-E-tag pAb
- PM071  Anti-Calmodulin Binding Protein-tag pAb
- M211-3  A Anti-Strep-tag II mAb (4F1)
- M214-3  Anti-mini-AID-tag mAb (1E4)
- M214-7  Anti-mini-AID-tag mAb-HRP-DirecT (1E4)

**Protein Purification Kits**

- 3305  c-Myc-tagged Protein MILD PURIFICATION KIT
- 3340  c-Myc-tagged Protein Magnetic PURIFICATION KIT
- 3306  c-Myc-tagged Protein MILD PURIFICATION GEL with Elution Peptide (1 mL gel, 1 mg peptide)
- 3300-205  c-Myc tag peptide (EQKLISEEDL) (5 mg)
- 3325  DDDDK-tagged Protein Magnetic PURIFICATION KIT
- 3343  DDDDK-tagged Protein Magnetic PURIFICATION KIT
- 3325-205  DDDDK-tag peptide (5 mg)
- 3326  DDDDK-tagged Protein PURIFICATION GEL with Elution Peptide (1 mL gel, 5 mg peptide)
- 3327  DDDDK-tagged Protein PURIFICATION GEL with Elution Peptide (5 mL gel, 25 mg peptide)
- 3328  DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
- 3326K  DDDDK-tagged Protein PURIFICATION CARTRIDGE
- 3320  HA-tagged Protein PURIFICATION KIT
- 3342  HA-tagged Protein Magnetic PURIFICATION KIT
- 3320-205  HA-tag peptide (10 mg)
- 3321  HA-tagged Protein Purification Gel (1 mL)
- 3310  His-tagged Protein PURIFICATION KIT
- 3310-205  His-tag peptide (10 mg)
- 3311  His-tagged Protein PURIFICATION GEL with Elution Peptide (1 mL gel, 10 mg peptide)
- 3317  V5-tagged Protein PURIFICATION KIT Ver.2
- 3341  V5-tagged Protein Magnetic PURIFICATION KIT
- 3318  V5-tagged Protein PURIFICATION GEL Ver.2
- 3315-205  V5-tag peptide

Other related antibodies and kits are also available. Please visit our website at [http://ruo.mbl.co.jp](http://ruo.mbl.co.jp)
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/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). 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-HPD-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 times).
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-HPD-DirecT (code: M185-7)