

Smart-IP Series

# Anti-HA-tag mAb-Magnetic Agarose

<b>CODE No.</b>	M132-10
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	5D8
<b>ISOTYPE</b>	Mouse IgG1 $\kappa$
<b>QUANTITY</b>	20 tests (Gel: 200 $\mu$ L)
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	Carrier protein conjugated synthetic peptide, YPYDVPDYA (HA-tag)
<b>REACTIVITY</b>	This antibody reacts with N-terminal and C-terminal HA-tagged proteins.
<b>FORMULATION</b>	400 $\mu$ g of antibody is covalently coupled to 200 $\mu$ L of magnetic agarose gel and provided as 400 $\mu$ L gel slurry suspended in PBS/0.1% ProClin 150
<b>STORAGE</b>	This gel slurry is stable for one year from the date of purchase when stored at 4°C.

## APPLICATION-CONFIRMED

Immunoprecipitation 10  $\mu$ L of gel/400  $\mu$ L of cell extract from  $1 \times 10^6$  cells

## REFERENCES

- 1) Surve, S., *et al.*, *Eukaryot. Cell* **11**, 183-193 (2012)
- 2) Mukai, R. and Ohshima, T., *Biochem. Biophys. Res. Commun.* **409**, 328-332 (2011)
- 3) Ohshima, T., *et al.*, *J. Cell Biochem.* **111**, 187-194 (2010)

Clone 5D8 is used in these references.

For more information, please visit our web site <http://ruo.mbl.co.jp/>

## RELATED PRODUCTS

### Smart-IP series

3190	Magnetic Rack
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
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)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

### Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-Direct (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-Direct (1G9)
PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-Direct (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
M180-A48	Anti-HA-tag mAb-Alexa Fluor® 488 (TANA2)
M180-A59	Anti-HA-tag mAb-Alexa Fluor® 594 (TANA2)
M180-A64	Anti-HA-tag mAb-Alexa Fluor® 647 (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-7	Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 µL)

M192-6	Anti-Myc-tag mAb-Biotin (My3)
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 (polyclonal) (0.1 mL)
D291-3	Anti-His-tag mAb (OGHis) (200 µL)
D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-Direct (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
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 (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-Direct (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM022	Anti-GST-tag pAb (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti-β-galactosidase mAb (5A3)
PM049	Anti-β-galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)

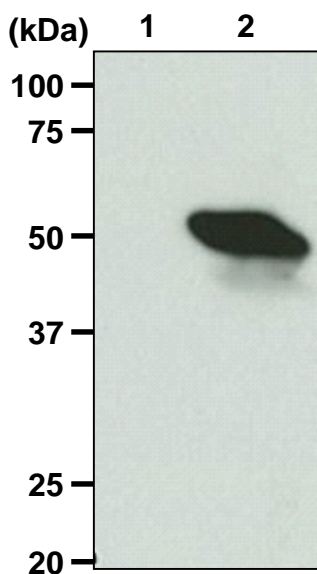
### Protein Purification Kits

3320	HA-tagged Protein PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)
3325	DDDDK-tagged Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3325-205	DDDDK-tag peptide (1 mg x 5)
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE (1 mL x 1)
3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3310	His-tagged Protein PURIFICATION KIT
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3317	V5-tagged Protein PURIFICATION KIT Ver.2
3318	V5-tagged Protein PURIFICATION GEL Ver. 2 (1 mL)

Other related antibodies and kits are also available.  
Please visit our website at <http://ruo.mbl.co.jp/>

### **Immunoprecipitation**

- 1) Wash  $1 \times 10^6$  cells 3 times with PBS and suspend them in 400  $\mu$ 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  $\mu$ 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) Wash the beads 4 times with 1 mL of cold Lysis buffer (place the tube on the magnetic rack for a few seconds).
- 7) Resuspend the magnetic beads in 50  $\mu$ L of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
- 8) Load 5  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> 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.
- 10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 11) Incubate the membrane with 1:10,000 of Anti-HA-tag mAb-HRP-Direct (MBL; code no. M180-7) 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.)
- 12) Wash the membrane with PBS-T (0.05% Tween-20 in PBS) (5 min. x 3 times).
- 13) 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.
- 14) 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 HA-tagged IκB***

Lane 1: Parental cell (293T)  
Lane 2: HA-tagged IκB/293T

Immunoblotted with Anti-HA-tag mAb-HRP-Direct (MBL; code no. M180-7)