

Smart-IP Series

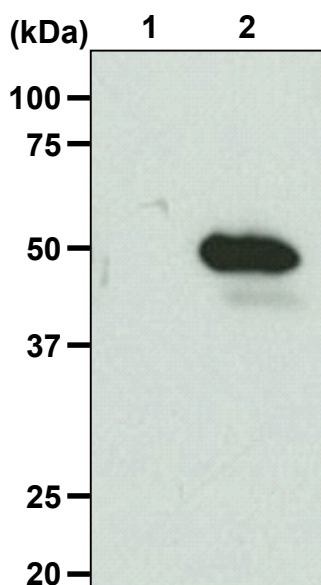
Anti-HA-tag mAb-Magnetic Agarose

CODE No.	M180-10
CLONALITY	Monoclonal
CLONE	TANA2
ISOTYPE	Mouse IgG2b κ
QUANTITY	20 tests (Gel: 200 μ L)
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, YPYDVPDYA (HA-tag)
REACTIVITY	This antibody reacts with N-terminal and C-terminal HA-tagged proteins.
FORMULATION	400 μ g of antibody is covalently coupled to 200 μ L of magnetic agarose gel and provided as 400 μ L 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	
<u>Immunoprecipitation</u>	10 μ L of gel/400 μ L of cell extract from 1×10^6 cells
REFERENCES	1) Ueyama, T., <i>et al.</i> , <i>J. Biol. Chem.</i> 290 , 6495-6506 (2015) [IP] 2) Ueyama, T., <i>et al.</i> , <i>J. Immunol.</i> 191 , 2560-2569 (2013) [IP]

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) 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 μ 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 μ 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 in methanol at 1 mA/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20%). 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) 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 I κ B

Lane 1: Parental cell (293T)
Lane 2: HA-tagged I κ B/293T

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

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