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

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 µL)
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 µg of antibody is covalently coupled to 200 µL of magnetic agarose gel and provided as 400 µL 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

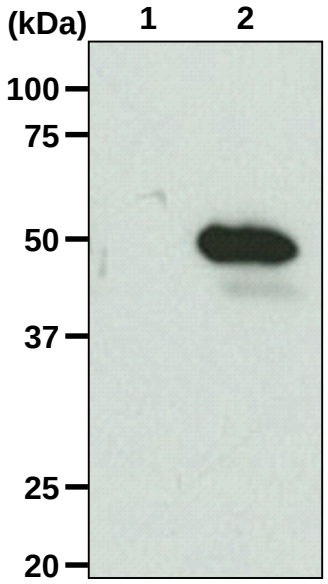
Immunoprecipitation	10 µL of gel/400 µL of cell extract from 1 x 10^6 cells

REFERENCES

1) Ueyama, T., *et al., J. Biol. Chem.* **290**, 6495-6506 (2015) [IP]
2) Ueyama, T., *et al., J. Immnol.* **191**, 2560-2569 (2013) [IP]

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) 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 µL of Laemmli's sample buffer, boil for 3 min., and place the tube on the magnetic rack for a few seconds.
8) Load 5 µL 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) membra methanol ne at 1 mA/cm^2 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 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)

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