

Smart-IP Series

Anti-His-tag mAb-Magnetic Beads

CODE No.	D291-11
CLONALITY	Monoclonal
CLONE	OGHis
ISOTYPE	Mouse IgG2a κ
QUANTITY	20 tests (Slurry: 1 mL)
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	6xHis-tagged protein
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal His-tagged proteins.
FORMULATION	1 mL PBS/0.1% BSA/0.09% NaN ₃
	*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
STORAGE	This beads suspension is stable for one year from the date of purchase when stored at 4°C. If bead agglomeration is observed, please disperse the agglomerations by careful pipetting. *In particular, please check the inner wall of the vial and cap.
APPLICATION-CONFIRMED	
<u>Immunoprecipitation</u>	50 μ L of beads slurry/sample
	*The purification capacity of Anti-His-tag mAb-Magnetic Beads varies depending upon the characteristics of a His-tagged protein. For example, 50 μ L of beads slurry bounds ≥ 1.5 μ g of a His-tagged protein (32 kDa).
REFERENCE	1) Ogawa, M., <i>et al.</i> , <i>Molecules</i> 23 , E1745 (2018) [IP]

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

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

Immunoprecipitation

- 1) Wash 1×10^7 cells 3 times with PBS and suspend with 1 mL of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% NP-40] containing appropriate protease inhibitors.
- 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 300 μ L of the supernatant prepared in step 2). Mix well and incubate with gentle agitation for 1 hr. 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 Wash buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] 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 20 μ L of Laemmli's sample buffer, boil for 5 min., and place the tube on the magnetic rack for a few seconds.
- 11) Load 20 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
- 12) Visualize the protein bands by CBB staining.



Immunoprecipitation of His-tagged protein

Sample: His-tagged β -galactosidase/293T whole cell lysate

Lane 1: Input (5 μ L/lane)

Lane 2: Post-IP beads of Anti-His-tag mAb-Magnetic Beads (MBL, code no. D291-11)