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

Anti-mini-AID-tag mAb

CODE No.	M214-3
CLONALITY	Monoclonal
CLONE	1E4
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 µL, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	17 aa sequence of Auxin Inducible Degron internal region (mini-AID-tag).
FORMULATION	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Western blotting	1-5 µg/mL for chemiluminescence detection system
Immunoprecipitation	5 µg/sample
Immunocytochemistry	5 µg/mL

REFERENCES

1) Davidson, L., *et al.*, *Cell Rep.* **26**, 2779-2791.e5 (2019) [WB]
2) Shen, E. Z., *et al.*, *Cell.***172**, 937-951.e18 (2018) [WB]
3) Schuller, A.P., *et al.*, *Mol. Cell.* **66**, 194-205.e5 (2017) [WB]
4) Natsume, T., *et al.*, *Genes Dev.* **31**, 816-829 (2017) [WB]
5) Natsume, T., *et al.*, *Cell Rep*. **15**, 210-218 (2016) [WB]
6) Nishimura, K. and Kanemaki, M. T., *Curr. Protoc. Cell Biol.* **64**, 20.9.1-20.9.16 (2014)
7) Kubota, T., *et al.*, *Mol. Cell* **50**, 273-280 (2013)
8) Nishimura, K., *et al.*, *Nat. Methods* **6**, 917-922 (2009)

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RELATED PRODUCTS

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)
561	Anti-HA-tag pAb (polyclonal) (100 µL)
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) (100 µL)
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)
D291-A48	Anti-His-tag mAb-Alexa Fluor® 488 (OGHis)
D291-A59	Anti-His-tag mAb-Alexa Fluor® 594 (OGHis)
D291-A64	Anti-His-tag mAb-Alexa Fluor® 647 (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)
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)

Smart-IP series

3190	Magnetic Rack
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)
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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)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

Other related antibodies and kits are also available.
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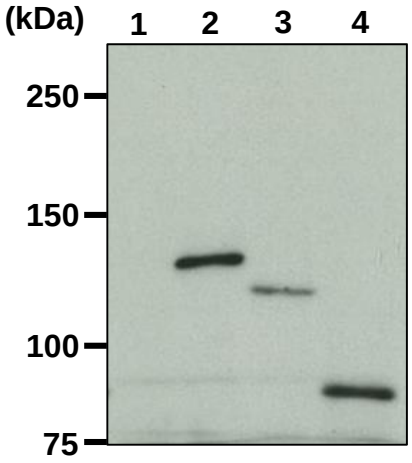
SDS-PAGE & Western blotting

1) Mix 600 μL of *E. coli* or *S. cerevisiae* culture into 1 mL of Laemmli's sample buffer, 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) Boil the samples for 5 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (5% or 12.5% acrylamide) for electrophoresis.
4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 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.
5) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
7) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T (5 min. x 3 times).
9) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
10) Wash the membrane with PBS-T (5 min. x 3 times).
11) 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.
12) Expose to an X-ray film in a dark room for 1-10 min. Develop the film as usual. The condition for exposure and development may vary.

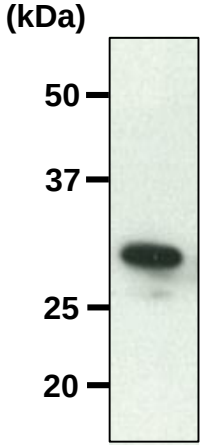


Western blot analysis of mini-AID-tagged proteins

Lane 1: *S. cerevisiae*
Lane 2: AID-tagged Mcm4/*S. cerevisiae*
Lane 3: mini-AID-tagged Mcm4/*S. cerevisiae*
Lane 4: 3 x mini-AID-tagged Mcm10/*S. cerevisiae*

Acrylamide gel: 5%
Exposure time: 10 min.

Immunoblotted with Anti-mini-AID-tag mAb (M214-3)



Western blot analysis of AID (full-length)

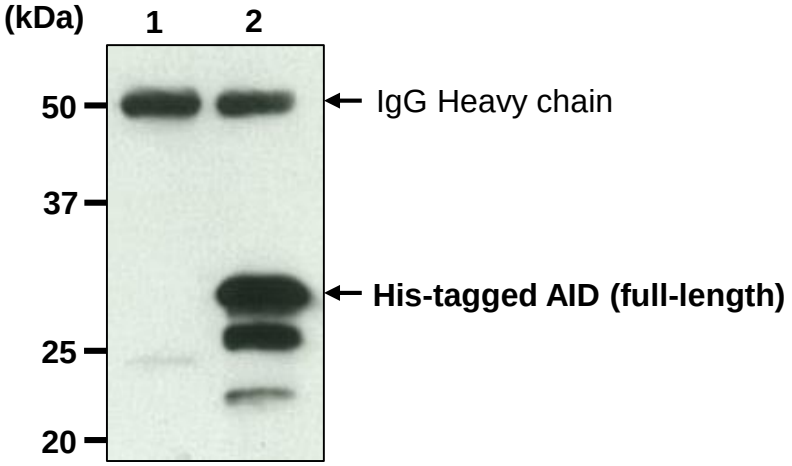
Sample: His-tagged AID (full-length)/*E. coli* (2.5 μL/lane)
Acrylamide gel: 12.5%
Exposure time: 1 min.

Immunoblotted with Anti-mini-AID-tag mAb (M214-3)

Samples were kindly provided by Dr. Masato Kanemaki.
(Molecular Function Laboratory, National Institute of Genetics)

Immunoprecipitation

1) Resuspend 1 mL *E. coli* culture with 1 mL of ice-cold Extraction buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors, then sonicate the cell suspension for 15 sec.
2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
3) Mix 20 µL of 50% protein A agarose beads slurry resuspended in 300 µL of Extraction buffer with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
4) Wash the beads 1 time with 1 mL of Extraction buffer.
5) Add 300 µL of cell lysate (prepared sample from step 2)), then incubate with gentle agitation for 1 hr. at 4°C.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Resuspend the agarose with 1 mL of Extraction buffer.
8) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
9) Repeat steps 6)-8) 3 times.
10) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
11) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
12) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 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) overnight at 4°C.
14) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
15) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
16) Wash the membrane with PBS-T (5 min. x 3 times).
17) Incubate the membrane with the 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
18) Wash the membrane with PBS-T (5 min. x 3 times).
19) 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.
20) Expose to an X-ray film in a dark room for 1 min. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of AID (full-length)

Sample: His-tagged AID (full-length)/*E. coli*
Lane 1: Mouse IgG2a (M076-3)
Lane 2: Anti-mini-AID-tag mAb (M214-3)

Immunoblotted with M214-3

Sample was kindly provided by Dr. Masato Kanemaki.
(Molecular Function Laboratory, National Institute of Genetics)