

Anti-mini-AID-tag mAb

CODE No.	M214-3
CLONALITY	Monoclonal
CLONE	1E4
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 μ L, 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

<u>Western blotting</u>	1-5 μ g/mL
<u>Immunoprecipitation</u>	5 μ g/sample
<u>Immunocytochemistry</u>	5 μ g/mL

REFERENCES

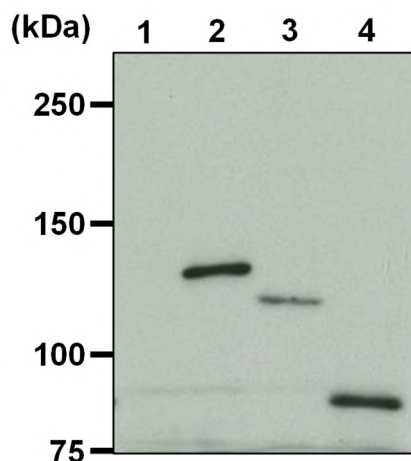
- 1) Davidson, L., *et al.*, *Cell Rep.* **26**, 2779-2791.e5 (2019) [WB]
- 2) Shen, E. Z., *et al.*, *Cell.* **172**, 937-951.e18 (2018) [WB]
- 3) Schuller, A.P., *et al.*, *Mol. Cell.* **66**, 194-205.e5 (2017) [WB]
- 4) Natsume, T., *et al.*, *Genes Dev.* **31**, 816-829 (2017) [WB]
- 5) Natsume, 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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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

- 1) Mix 600 μ L 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 μ L 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/cm² for 1 hr. in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% methanol). 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).
- 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).
- 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).
- 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.

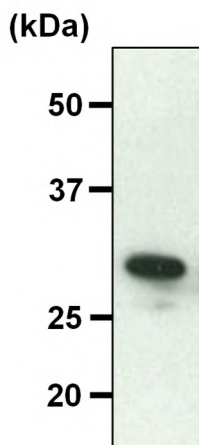


Western blotting analysis of mini-AID-tagged proteins

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 blotting analysis of AID (full-length)

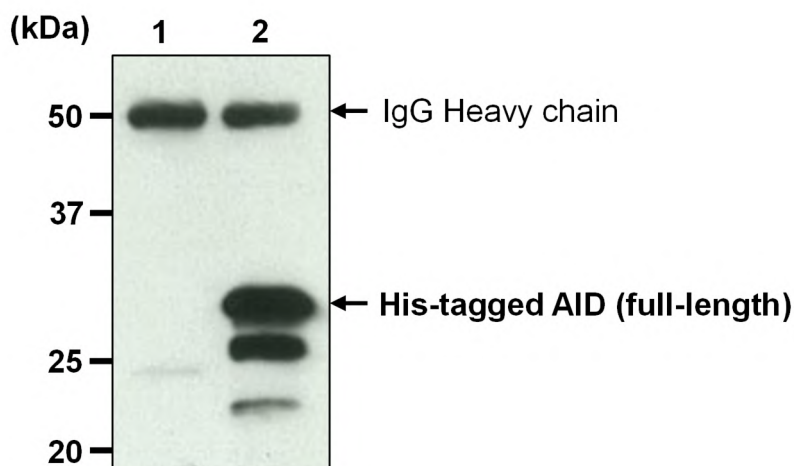
Sample: His-tagged AID (full-length)/*E. coli* (2.5 μ L/lane)
Acrylamide gel: 12.5%
Exposure time: 1 min.

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