

# Anti-mini-AID-tag mAb

|                    |                                                                                               |
|--------------------|-----------------------------------------------------------------------------------------------|
| <b>CODE No.</b>    | M214-3                                                                                        |
| <b>CLONALITY</b>   | Monoclonal                                                                                    |
| <b>CLONE</b>       | 1E4                                                                                           |
| <b>ISOTYPE</b>     | Mouse IgG2a $\kappa$                                                                          |
| <b>QUANTITY</b>    | 100 $\mu$ L, 1 mg/mL                                                                          |
| <b>SOURCE</b>      | Purified IgG from hybridoma supernatant                                                       |
| <b>IMMUNOGEN</b>   | 17 aa sequence of Auxin Inducible Degron internal region (mini-AID-tag).                      |
| <b>FORMULATION</b> | PBS containing 50% Glycerol (pH 7.2). No preservative is contained.                           |
| <b>STORAGE</b>     | 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 $\mu$ g/mL   |
| <u>Immunoprecipitation</u> | 5 $\mu$ g/sample |
| <u>Immunocytochemistry</u> | 5 $\mu$ g/mL     |

## REFERENCES

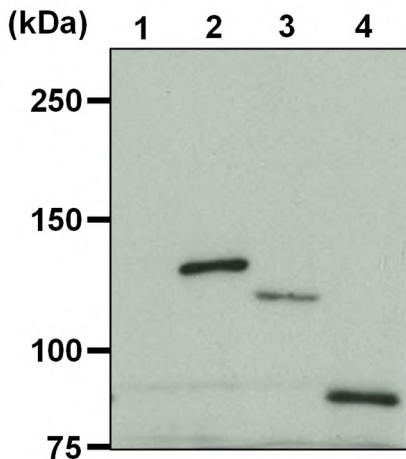
- 1) Davidson, L., *et al.*, *Cell Rep.* **26**, 2779-2791.e5 (2019) [WB]
- 2) Shen, E. Z., *et al.*, *Cell.* **172**, 937-951.e18 (2018) [WB]
- 3) Schuller, A.P., *et al.*, *Mol. Cell.* **66**, 194-205.e5 (2017) [WB]
- 4) Natsume, T., *et al.*, *Genes Dev.* **31**, 816-829 (2017) [WB]
- 5) Natsume, 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  $\mu$ 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  $\mu$ 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/cm<sup>2</sup> 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.

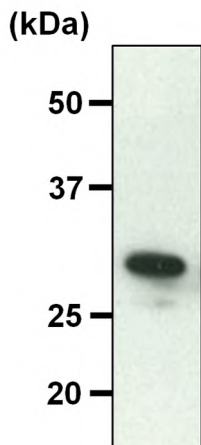


#### **Western blotting analysis of mini-AID-tagged proteins**

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)**

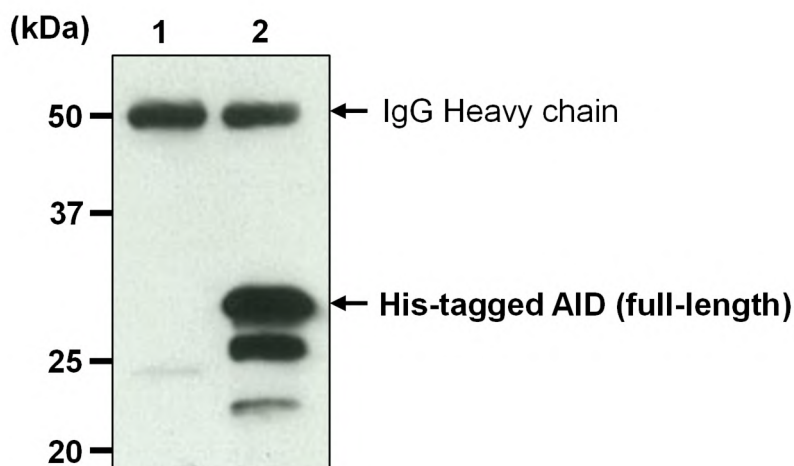
Sample: His-tagged AID (full-length)/*E. coli* (2.5  $\mu$ L/lane)  
Acrylamide gel: 12.5%  
Exposure time: 1 min.

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/cm<sup>2</sup> 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)