

## Anti-Ash-tag mAb

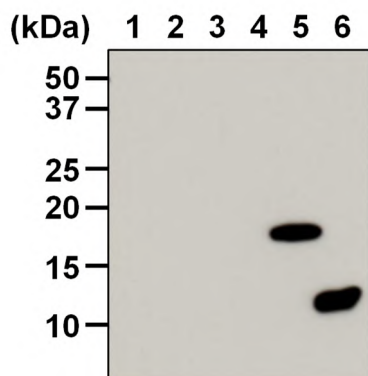
<b>CODE No.</b>	M223-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	FLP1C15-2
<b>ISOTYPE</b>	Mouse IgG1
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>REACTIVITY</b>	This antibody reacts with proteins expressed by Ash-MCL and Ash-MNL Ver.2 (components of code no. AM8011M and AM8012M) but not with Ash-MNLs including lentiviral vectors. (components of code no. SI-8010, SI-8011, SI-8020, SI-8021)
<b>FORMULATION</b>	PBS containing 50% glycerol. 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.
<b>APPLICATION-CONFIRMED</b>	
<u>Western blotting</u>	5 $\mu$ g/mL

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

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

### **SDS-PAGE & Western blotting**

- 1) Wash  $1.2 \times 10^6$  cells 3 times with PBS. and suspend them in 120  $\mu$ L of 1 x 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 sample for 10 min. and centrifuge. Load 10  $\mu$ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (15% 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% MeOH). See the manufacturer's manual for precise transfer procedure.
- 5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 6) 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.)
- 7) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 8) Incubate the membrane with the 1:5,000 of 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.
- 9) Wash the membrane with PBS-T (5 min. x 3 times).
- 10) Wipe excess buffer on the membrane, and 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.
- 11) 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.



#### ***Western blot analysis of Ash-tagged protein from HEK293T transfectant***

- Lane 1: pMonti-Red-MCL
- Lane 2: pMonti-Red-MNL
- Lane 3: phAG-MCL
- Lane 4: phAG-MNL
- Lane 5: pAsh-MCL
- Lane 6: pAsh-MNL Ver.2

Immunoblotted with Anti-Ash-tag mAb (M223-3)