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

Amalgaam MBL

# Anti-Ash-tag mAb

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| **CODE No.** | M223-3 |
| **CLONALITY** | Monoclonal |
| **CLONE** | FLP1C15-2 |
| **ISOTYPE** | Mouse IgG1 |
| **QUANTITY** | 100 µL, 1 mg/mL |
| **SOURCE** | Purified IgG from hybridoma supernatant |
| **REACTIVITY** | 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) |
| **FORMULATION** | PBS containing 50% glycerol. No preservative is contained. |
| **STORAGE** | This antibody solution is stable for one year from the date of purchase when stored at -20°C. |

**APPLICATION-CONFIRMED**

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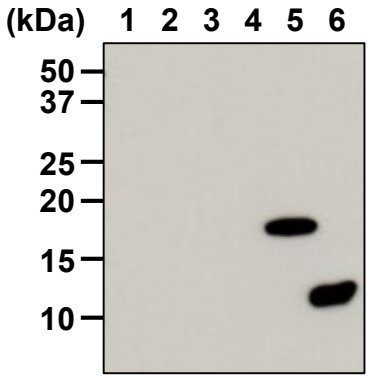
| Western blotting | 5 µg/mL |

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 x $10^6$ cells 3 times with PBS. and suspend them in 120 μL 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 μL 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/$cm^2$ 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 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)