

Anti-Myc-tag mAb-HRP-Direct

CODE No.	M192-7
CLONALITY	Monoclonal
CLONE	My3
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μ L
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, EQKLISEEDL (Myc-tag)
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal Myc-tagged proteins.
FORMULATION	PBS/Preservative/Stabilizer
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATION-CONFIRMED

Western blotting 1:10,000

REFERENCES

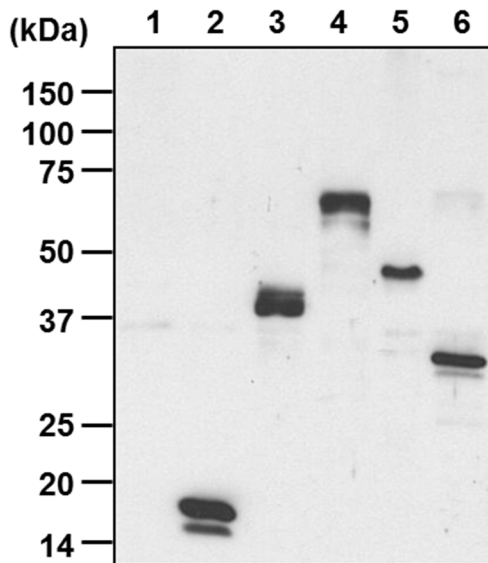
- 1) Nakazono, A., *et al.*, *J. Biol. Chem.* **293**, 14758-14774 (2018) [WB]
- 2) Hu, L., *et al.*, *Plant Cell* **29**, 3157-3185 (2017) [WB]
- 3) Miyazawa-Onami, M., *et al.*, *EMBO Rep.* **18**, 1752-1761 (2017) [WB]
- 4) Qiu, Y., *et al.*, *Immunity* **46**, 992-1004.e5 (2017) [WB]

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

SDS-PAGE & Western blotting

- 1) Wash 1×10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer.
- 2) Boil the samples for 3 min. and centrifuge at 12,000 xg for 5 min. Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) and carry out electrophoresis.
- 3) 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.
- 4) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
- 5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3).
- 6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATION** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 7) Wash the membrane with PBS-T (5 min. x 3).
- 8) 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.
- 9) 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 blotting analysis of Myc-tagged protein

- Lane 1: 293T cell lysate (negative control)
- Lane 2: N-terminal Myc-tagged protein A/293T
- Lane 3: C-terminal Myc-tagged protein B/293T
- Lane 4: C-terminal Myc-tagged protein C/293T
- Lane 5: C-terminal Myc-tagged protein D/293T
- Lane 6: Internal Myc-tagged mAG purified protein (10 ng)

Immunoblotted with Anti-Myc-tag mAb-HRP-DirecT
(MBL, code no. M192-7)