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Anti-Myc-tag mAb-HRP-DirecT

CODE No.	M192-7
CLONALITY	Monoclonal
CLONE	My3
ISOTYPE	Mouse IgG2b κ
QUANTITY	100 μL
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

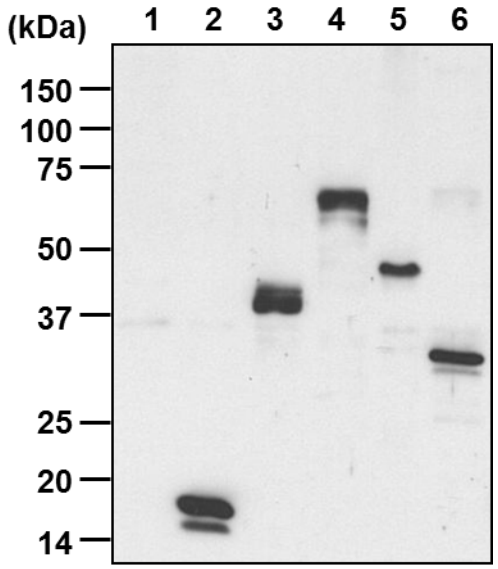
1) Nakazono, A., *et al., J. Biol. Chem.* **293**, 14758-14774 (2018) [WB]
2) Hu, L., *et al., Plant Cell* **29**, 3157-3185 (2017) [WB]
3) Miyazawa-Onami, M., *et al., EMBO Rep.* **18**, 1752-1761 (2017) [WB]
4) Qiu, Y., *et al., Immunity* **46**, 992-1004.e5 (2017) [WB]

For more information, please visit our web site **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 x 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 μL 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/cm^2 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)