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Not for use in diagnostic procedures.

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Anti-DDDDK-tag mAb-HRP-DirecT

CODE No.	M185-7
CLONALITY	Monoclonal
CLONE	FLA-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	200 μL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated DYKDDDDK peptide (DDDDK-tag)
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-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	1:2,000-1:5,000

REFERENCES

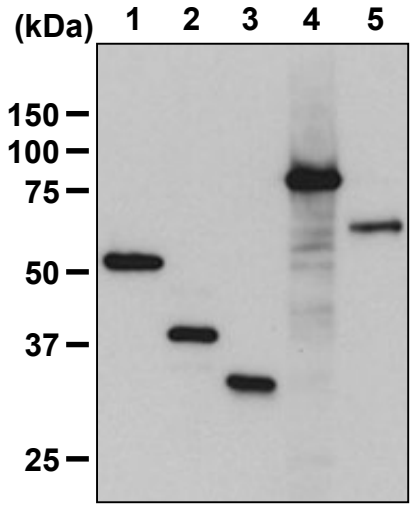
1) Zhang, X., *et al*., *Front. Microbiol.* **9**, 507 (2018) [WB]
2) Liu, Q., *et al*., *Nat. Commun.* **9**, 852 (2018) [WB]
3) Shibata, T., *et al*., *J. Biol. Chem*. **292**, 10723-10734 (2017) [WB]
4) Hiramatsu, H., *et al*., *Sci. Rep*. **7**, 889 (2017) [WB]
5) Ichida, Y., *et al*., *PLoS One* **10**, e0139274 (2015) [WB]
6) Lin, Q., *et al*., *Nat. Commun.* **6**, 7981 (2015) [WB]
7) Takahashi, RU., *et al*., *Nat. Commun.* **6**, 7318 (2015) [WB]
8) Takahashi, RU., *et al*., *Sci. Rep*. **3**, 2474 (2013) [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, then sonicate briefly (up to 10 sec.).
2) Boil the samples for 3 min. and centrifuge. Load 10 μL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for 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) overnight at 4°C.
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 DDDDK-tagged protein

Lane 1: Met-N-terminal DDDDK-tagged protein A
Lane 2: N-terminal DDDDK-tagged protein B/293T
Lane 3: Internal DDDDK-tagged protein C
Lane 4: Internal DDDDK-tagged protein D/293T
Lane 5: C-terminal DDDDK-tagged protein E/293T

Immunoblotted with Anti-DDDDK-tag mAb-HRP-DirecT
(MBL, code no. M185-7)1:5,000