

# Anti-DDDDK-tag mAb-HRP-Direct

<b>CODE No.</b>	M185-7
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	FLA-1
<b>ISOTYPE</b>	Mouse IgG2a $\kappa$
<b>QUANTITY</b>	200 $\mu$ L
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH conjugated DYKDDDDK peptide (DDDDK-tag)
<b>REACTIVITY</b>	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged proteins.
<b>FORMULATION</b>	PBS/Preservative/Stabilizer
<b>STORAGE</b>	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

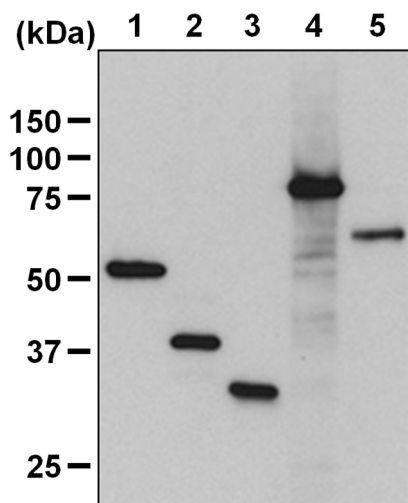
- 1) Zhang, X., *et al.*, *Front. Microbiol.* **9**, 507 (2018) [WB]
- 2) Liu, Q., *et al.*, *Nat. Commun.* **9**, 852 (2018) [WB]
- 3) Shibata, T., *et al.*, *J. Biol. Chem.* **292**, 10723-10734 (2017) [WB]
- 4) Hiramatsu, H., *et al.*, *Sci. Rep.* **7**, 889 (2017) [WB]
- 5) Ichida, Y., *et al.*, *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]

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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  $1 \times 10^6$  cells 3 times with PBS and suspend 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  $\mu$ L 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<sup>2</sup> 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