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

Anti-DDDDK-tag mAb-Biotin

CODE No.	M185-6
CLONALITY	Monoclonal
CLONE	FLA-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	50 μL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated synthetic peptide, DYKDDDDK
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged (DYKDDDDK) proteins.
FORMULATION	PBS (pH 7.2) containing 1% BSA and 0.09% NaN_3

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE This antibody solution is stable for one year from the date of purchase when stored at 4°C.

APPLICATIONS-CONFIRMED

Western blotting	1:2,000
Sandwich ELISA	1:2,000 for chemiluminescence detection system

RELATED PRODUCTS

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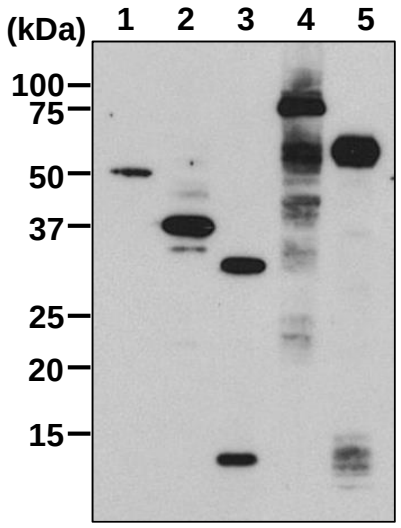
The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

SDS-PAGE & Western blotting

1) Prepare samples described as below:
   [Transfectant] 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.).
   [Recombinant protein] Mix the samples with equal volume of Laemmli's sample buffer.
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% MeOH). 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 times).
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 (5 min. x 3 times).
8) Incubate the membrane with Streptavidin-HRP 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 3 min. Develop the film as usual. The condition for exposure and development may vary.



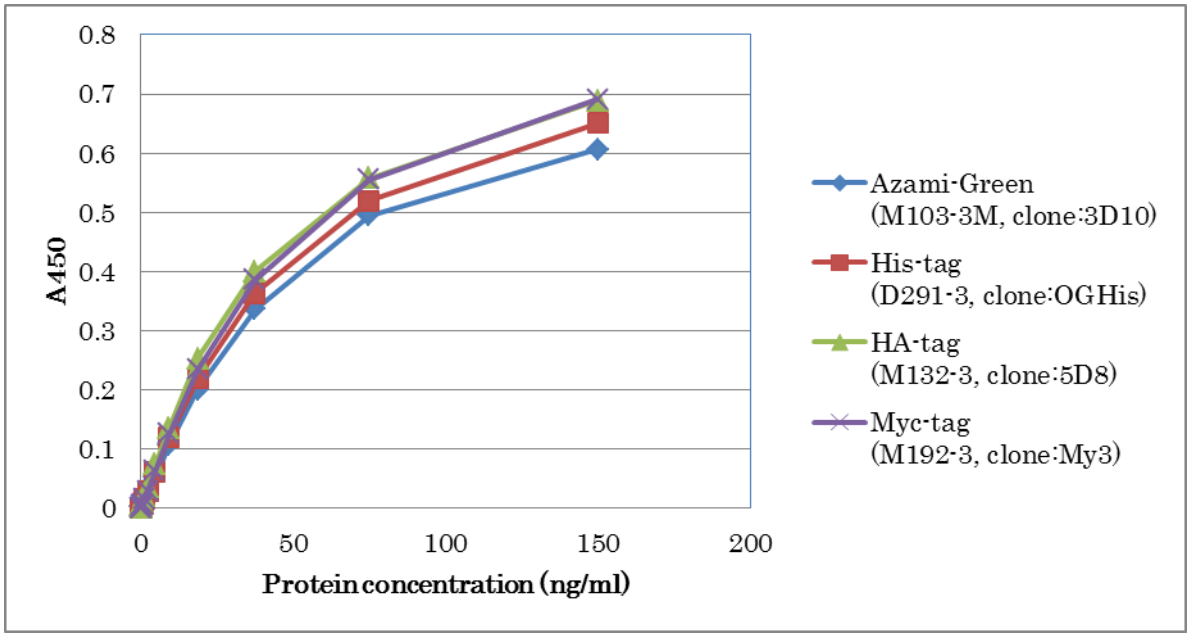
Western blot analysis of DDDDK-tagged protein

Lane 1: N-terminal Met-DDDDK-tagged protein A
Lane 2: N-terminal DDDDK-tagged protein B/HEK293T
Lane 3: Internal DDDDK-tagged protein C
Lane 4: 3x DDDDK-tagged protein D/HEK293T
Lane 5: C-terminal DDDDK-tagged protein E/HEK293T

Immunoblotted with Anti-DDDDK-tag mAb-Biotin (M185-6)

Sandwich ELISA

1) Add 100 µL/well of 5 µg/mL capture antibody diluted 0.1 M Carbonate buffer (pH 9.6) to the 96-well microplate. Incubate for 1 hr. at room temperature.
2) Wash the plate with PBS (1 time).
3) Add 200 µL/well of Blocking Buffer (1% BSA/5% Sucrose/0.15% Proclin150/PBS). Incubate for 1 hr. at room temperature.
4) Discard the Blocking Buffer. Add 100 µL/well of epitope-tagged control protein (His-DDDDK-V5-HA-Myc-monomeric Azami-Green) in Assay diluent (1% BSA/0.1% Tween-20/0.15% Proclin150/PBS) to each well.
5) Incubate for 1 hr. at room temperature.
6) Wash the plate with PBS-T [0.05% Tween-20 in PBS] (4 times).
7) Add 100 µL/well of Anti-DDDDK-tag mAb-Biotin (MBL; code no. M185-6) diluted with Assay diluent as suggested in the **APPLICATION**. Incubate for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the plate with PBS-T (4 times).
9) Add 100 µL/well of 1:40,000 streptavidin-HRP (GE Healthcare; code no. RPN4401) diluted with SA-HRP diluent (20 mM HEPES/1% BSA/0.135 M NaCl). Incubate for 30 min. at room temperature.
10) Wash the plate with PBS-T (4 times).
11) Add 100 µL/well of substrate solution (ex. TMB). Incubate for 30 min. at room temperature.
12) Add 100 µL/well of stop solution (ex. 1 M H_2SO_4).
13) Read at A450 /620.



ELISA for measurement of DDDDK-tagged protein

Sample:
His-DDDDK-V5-HA-Myc-monomeric Azami-Green
Capture antibody:
Anti-Azami-Green mAb (MBL; code no. M103-3M)
Anti-His-tag mAb (MBL; code no. D291-3)
Anti-HA-tag mAb (MBL; code no. M132-3)
Anti-Myc-tag mAb (MBL; code no. M192-3)
Detector antibody:
Anti-DDDDK-tag mAb-Biotin (MBL; code no. M185-6)