

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 μ L
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.
FORMURATION	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	
<u>Western blotting</u>	1:2,000 for chemiluminescence detection system
<u>Sandwich ELISA</u>	1:2,000 for chemiluminescence detection system

For more information, please visit our web site <http://ruo.mbl.co.jp/>



RELATED PRODUCTS

Antibodies

M192-3	Anti-Myc-tag mAb (My3) (200 µL)
M192-6	Anti-Myc-tag mAb-Biotin (My3)
M047-3	Anti-Myc-tag mAb (PL14)
M047-6	Anti-Myc-tag mAb-Biotin (PL14)
M047-7	Anti-Myc-tag mAb-HRP-Direct (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
M047-A48	Anti-Myc-tag mAb-Alexa Fluor® 488 (PL14)
M047-A59	Anti-Myc-tag mAb-Alexa Fluor® 594 (PL14)
M047-A64	Anti-Myc-tag mAb-Alexa Fluor® 647 (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
562-5	Anti-Myc-tag pAb (polyclonal) (0.5 mL)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-Direct (TANA2)
M180-A48	Anti-HA-tag mAb-Alexa Fluor® 488 (TANA2)
M180-A59	Anti-HA-tag mAb-Alexa Fluor® 594 (TANA2)
M180-A64	Anti-HA-tag mAb-Alexa Fluor® 647 (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-5	Anti-HA-tag pAb (polyclonal) (0.5 mL)
561-7	Anti-HA-tag pAb-HRP-Direct (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3S	Anti-DDDDK-tag mAb (FLA-1) (50 µL)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-A48	Anti-DDDDK-tag mAb-Alexa Fluor® 488 (FLA-1)
M185-A59	Anti-DDDDK-tag mAb-Alexa Fluor® 594 (FLA-1)
M185-A64	Anti-DDDDK-tag mAb-Alexa Fluor® 647 (FLA-1)
M185-6	Anti-DDDDK-tag mAb-Biotin (FLA-1)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
D291-3	Anti-His-tag mAb (OGHis) (200 µL)
D291-6	Anti-His-tag mAb-Biotin (OGHis)
D291-7	Anti-His-tag mAb-HRP-Direct (OGHis)
D291-8	Anti-His-tag mAb-Agarose (OGHis)
D291-A48	Anti-His-tag mAb-Alexa Fluor® 488 (OGHis)
D291-A59	Anti-His-tag mAb-Alexa Fluor® 594 (OGHis)
D291-A64	Anti-His-tag mAb-Alexa Fluor® 647 (OGHis)
M089-3	Anti-His-tag mAb (6C4)
M136-3	Anti-His-tag mAb (2D8)
PM032	Anti-His-tag pAb (polyclonal)
PM032-8	Anti-His-tag pAb-Agarose (polyclonal)
M167-3	Anti-V5-tag mAb (1H6)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-Direct (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-Direct (polyclonal)
M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-A48	Anti-GFP mAb-Alexa Fluor® 488 (RQ2)
D153-A59	Anti-GFP mAb-Alexa Fluor® 594 (RQ2)
D153-A64	Anti-GFP mAb-Alexa Fluor® 647 (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)

PM005	Anti-RFP pAb (polyclonal)
PM005-7	Anti-RFP pAb-HRP-Direct (polyclonal)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
PM070	Anti-E-tag pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)

Smart-IP series

3190	Magnetic Rack
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
D291-11	Anti-His-tag mAb-Magnetic Beads (OGHis)
D153-11	Anti-GFP mAb-Magnetic Beads (RQ2)
M165-11	Anti-RFP mAb-Magnetic Beads (3G5)
M167-11	Anti-V5-tag mAb-Magnetic Beads (1H6)
M198-9	Anti-E-tag mAb-Magnetic beads (21D11)
D058-9	Anti-Multi Ubiquitin mAb-Magnetic beads (FK2)
M075-11	Mouse IgG1 (isotype control)-Magnetic Beads
M076-11	Mouse IgG2a (isotype control)-Magnetic Beads
M077-11	Mouse IgG2b (isotype control)-Magnetic Beads
M081-11	Rat IgG2a (isotype control)-Magnetic Beads
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
D291-10	Anti-His-tag mAb-Magnetic Agarose (OGHis)
D153-10	Anti-GFP mAb-Magnetic Agarose (RQ2)
M165-10	Anti-RFP mAb-Magnetic Agarose (3G5)
M167-10	Anti-V5-tag mAb-Magnetic Agarose (1H6)
M198-10	Anti-E-tag mAb-Magnetic Agarose (21D11)

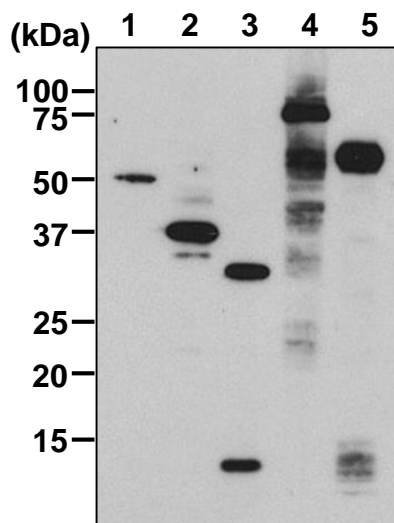
Protein Purification Kits

3305	c-Myc-tagged Protein MILD PURIFICATION KIT
3306	c-Myc-tagged Protein MILD PURIFICATION GEL (1 mL gel, 1 mg peptide)
3320	HA-tagged Protein PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)
3325	DDDDK-tagged Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3327	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel, 25 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3329	DDDDK-tagged Protein PURIFICATION GEL (25 mL gel)
3325-205	DDDDK-tag peptide (1 mg x 5)
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE (1 mL x 1)
3310	His-tagged Protein PURIFICATION KIT
3311	His-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3317	V5-tagged Protein PURIFICATION KIT Ver.2
3316	V5-tagged Protein PURIFICATION GEL Ver.2 (1 mL)

Other related antibodies and kits are also available.
Please visit our website at <http://ruo.mbl.co.jp/>

SDS-PAGE & Western blotting

- 1) Prepare samples described as below:
[Transfectant] Wash 1×10^6 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 μ 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/cm² 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.



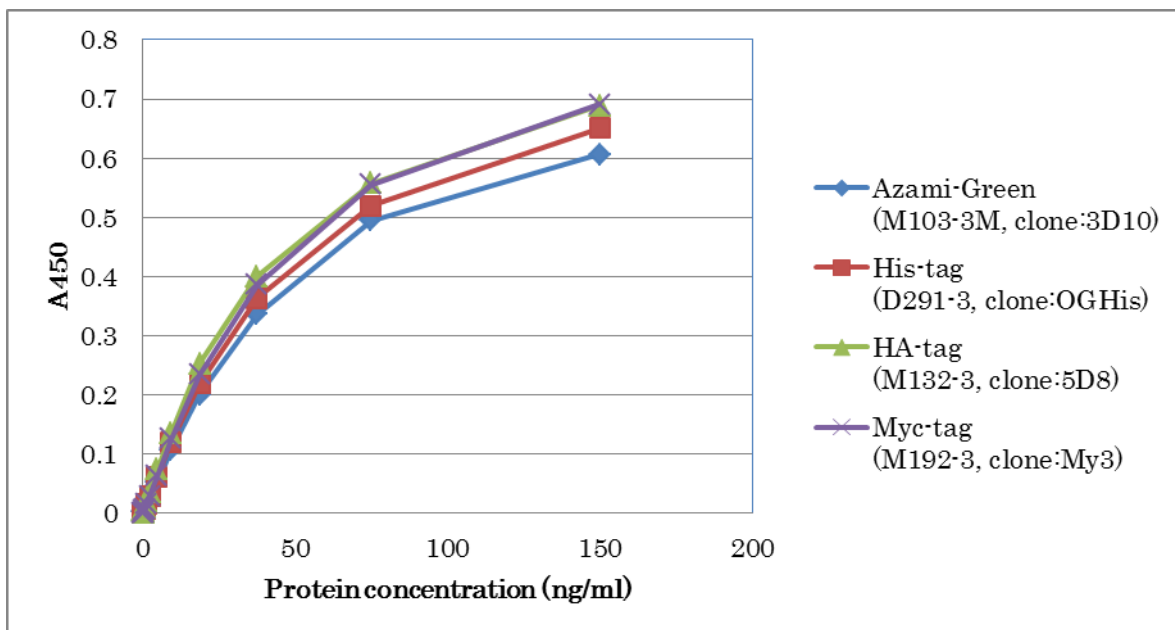
Western blot analysis of DDDDK-tagged protein

- 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 $\mu\text{g}/\text{mL}$ 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)