

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



Anti-DDDDK-tag mAb-Alexa Fluor[®] 647

CODE No.	M185-A64
CLONALITY	Monoclonal
CLONE	FLA-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	100 μ L, 1 mg/mL
SOURCE	Purified IgG from hybridoma supernatant
IMMUNOGEN	KLH conjugated DYKDDDDK peptide
REACTIVITY	This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged (DYKDDDDK) proteins.
FORMULATION	PBS containing 1% BSA and 0.1% ProClin 150.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at 4°C.
APPLICATIONS-CONFIRMED	
<u>Immunocytochemistry</u>	0.5-1 μ g/mL
<u>Flow cytometry</u>	0.5 μ g/mL

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

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Alexa Fluor[®] is a registered trademark of Molecular Probes, Inc.



MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL <http://ruo.mbl.co.jp/>
e-mail support@mbi.co.jp, TEL 052-238-1904

RELATED PRODUCTSAntibodies

M185-3S	Anti-DDDDK-tag mAb (FLA-1) (50 µL)
M185-3L	Anti-DDDDK-tag mAb (FLA-1) (1 mL)
M185-3LL	Anti-DDDDK-tag mAb (FLA-1) (1 mL x 5)
M185-6	Anti-DDDDK-tag mAb-Biotin (FLA-1)
M185-7	Anti-DDDDK-tag mAb-HRP-Direct (FLA-1)
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)
PM020	Anti-DDDDK-tag pAb (polyclonal)
PM020-7	Anti-DDDDK-tag pAb-HRP-Direct (polyclonal)
PM020-8	Anti-DDDDK-tag pAb-Agarose (polyclonal)
M192-3	Anti-Myc-tag mAb (My3) (200 µL)
M047-3	Anti-Myc-tag mAb (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)
M180-7	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)
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
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-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
M185-11	Anti-DDDDK-tag mAb-Magnetic Beads (FLA-1)
M047-11	Anti-Myc-tag mAb-Magnetic Beads (PL14)
M132-11	Anti-HA-tag mAb-Magnetic Beads (5D8)
M180-11	Anti-HA-tag mAb-Magnetic Beads (TANA2)
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)
D058-11	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
M185-10	Anti-DDDDK-tag mAb-Magnetic Agarose (FLA-1)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
M132-10	Anti-HA-tag mAb-Magnetic Agarose (5D8)
M180-10	Anti-HA-tag mAb-Magnetic Agarose (TANA2)
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 Kit

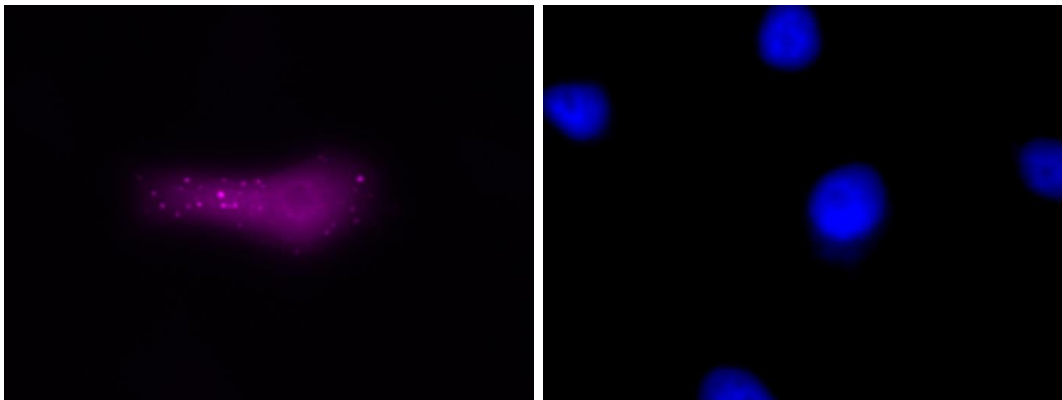
3325	DDDDK-tagged Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3327	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel, 25 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 mL gel)
3329	DDDDK-tagged Protein PURIFICATION GEL (25 mL gel)
3325-205	DDDDK-tag peptide (1 mg x 5)
3326K	DDDDK-tagged Protein PURIFICATION CARTRIDGE (1 mL x 1)
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)
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
3318	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/>

Immunocytochemistry

- 1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO₂ incubator for one night.
- 2) Remove the culture supernatant by careful aspiration.
- 3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
- 4) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the fixed cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 min. Take care not to touch the cells. Repeat another wash once more.
- 5) Immerse the slide in 0.2% Triton X-100/PBS for 10 min. at room temperature.
- 6) Wash the slide in a plenty of PBS as in the step 4).
- 7) Cover each cell with Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) for 5 min. at room temperature.
- 8) Add 200 µL of the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hr. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 9) Wash the slide in a plenty of PBS as in the step 4).
- 10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 11) Counter stain with DAPI for 5 min. at room temperature.
- 12) Wash the slide in a plenty of PBS as in the step 4).
- 13) Promptly add mounting medium onto the slide, then put a cover slip on it.



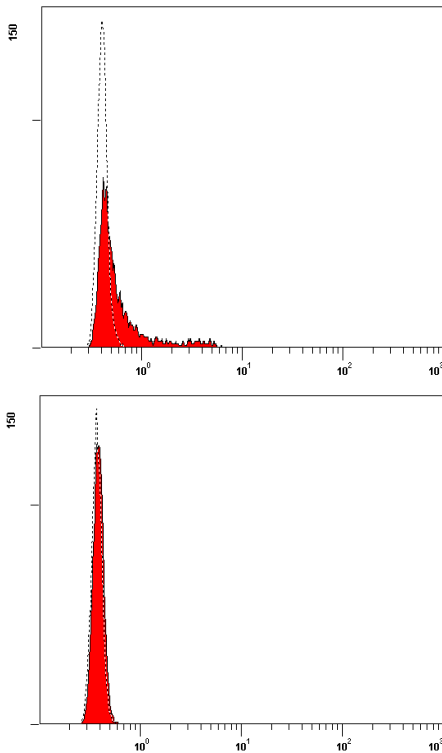
Immunocytochemical detection of DDDDK-tagged protein in HeLa

Magenta: M185-A64

Cyan: DAPI

Flow cytometric analysis for adherent cells

- 1) Detach the cells from culture dish.
- 2) Wash the cells 1 time with 1 mL of washing buffer [PBS containing 2% fetal calf serum (FCS)].
- 3) Add 200 μ L of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 10 min. at room temperature.
- 4) Wash the cells 2 times with 1 mL of washing buffer.
- 5) Add 200 μ L of PBS containing 0.2% Triton X-100 to the cell pellet after tapping. Mix well, then permeabilize the cells for 10 min. at room temperature.
- 6) Wash the cells 1 time with 1 mL of washing buffer.
- 7) Resuspend the cells with washing buffer (1.6×10^6 cells/mL).
- 8) Add 100 μ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 min. at room temperature (20~25°C). Remove supernatant by careful aspiration.
- 9) Add 20 μ L of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 min. at room temperature.
- 10) Add 40 μ L of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in the washing buffer. Mix well and incubate for 30 min. at room temperature.
- 11) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 min. at room temperature. Remove supernatant by careful aspiration. Repeat another wash once more.
- 12) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of DDDDK-tagged protein in HeLa

Closed: M185-A64

Open: Isotype control (M076-A64)

Upper: DDDDK-tagged protein in HeLa

Lower: Parental cell (HeLa)