

Anti-DDDDK-tag mAb

CODE No.	M185-3L
CLONALITY	Monoclonal
CLONE	FLA-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	1 mL, 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 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

<u>Western blotting</u>	0.1 μ g/mL for chemiluminescence detection system
<u>Immunoprecipitation</u>	2 μ g/sample
<u>Immunocytochemistry</u>	0.1 μ g/mL
<u>Flow cytometry</u>	0.05 μ g/mL

APPLICATION-REPORTED

<u>Chromatin Immunoprecipitation</u>	Reference 4)
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REFERENCES

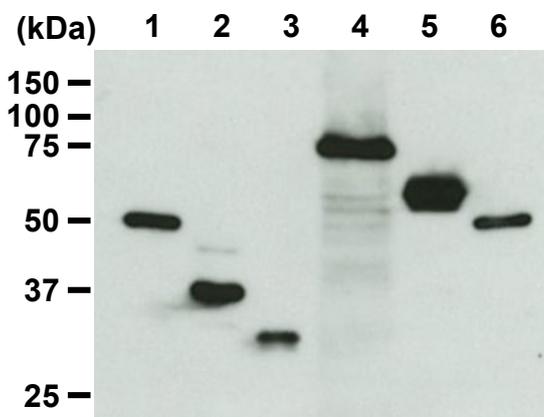
- 1) Nishida, K. M., *et al.*, *Nature* **555**, 260-264 (2018) [WB]
- 2) Tu, R., *et al.*, *Cell Death Dis.* **9**, 553 (2018) [WB, IP]
- 3) Yang, J., *et al.*, *PLoS Genet.* **13**, e1006975 (2017) [WB]
- 4) Lee, Y. K., *et al.*, *Cancer Lett.* **403**, 144-151 (2017) [WB, ChIP]
- 5) Song, K. H., *et al.*, *Cell Death Dis.* **8**, e2536 (2017) [WB, IP]
- 6) Feng, S., *et al.*, *J Biol Chem.* **291**, 21956-21962 (2016) [WB, IC]
- 7) Fan, L., *et al.*, *Oncotarget* **7**, 63887-63900 (2016) [WB]
- 8) Hossain, M. S., *et al.*, *PLoS One* **11**, e0150846 (2016) [WB]
- 9) Wu, T., *et al.*, *PLoS One* **11**, e0149361 (2016) [Co-IP]

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

SDS-PAGE & Western blotting

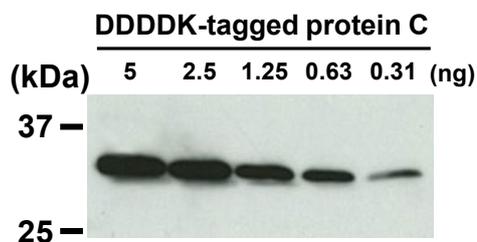
- 1) For transfected cells: wash 1×10^6 cells 3 times with PBS and suspends them in 1 mL of Laemmli's sample buffer, then sonicate briefly (up to 10 sec.).
For recombinant proteins: mix the samples with equal volume of Laemmli's sample buffer.
- 2) Boil the samples for 3 min. and centrifuge. Load 10 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) overnight at 4°C.
- 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 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) 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 1 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/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
- Lane 6: C-terminal DDDDK-tagged protein A

Immunoblotted with M185-3L

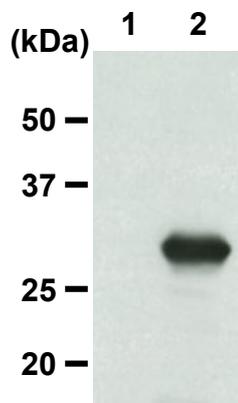


Western blot analysis of DDDDK-tagged protein C

Immunoblotted with M185-3L

Immunoprecipitation

- 1) Mix 20 μ L of 50% protein A agarose beads slurry resuspended in 300 μ L of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40] with primary antibody as suggested in the **APPLICATIONS**. Incubate with gently agitation for 1 hr. at room temperature.
- 2) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 3) Resuspend the agarose with 1 mL of IP buffer.
- 4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
- 5) Repeat steps 2)-4) 2 times.
- 6) Add 2 μ g of recombinant protein in 300 μ L of Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40], then incubate with gentle agitation for 1 hr. at room temperature.
- 7) Wash the beads 5 times with 1 mL of Lysis buffer.
- 8) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 2 min. and centrifuge.
- 9) Load 10 μ L of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 10) 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.
- 11) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 12) Incubate the membrane with 1:5,000 Anti-DDDDK-tag pAb-HRP-DirecT (MBL; code no. PM020-7) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
- 13) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
- 14) Wipe excess buffer on the membrane, and then incubate it with appropriate chemiluminescence reagent for 1 min.
- 15) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 16) Expose to an X-ray film in a dark room for 30 sec. Develop the film as usual. The condition for exposure and development may vary.



Immunoprecipitation of DDDDK-tagged GFP

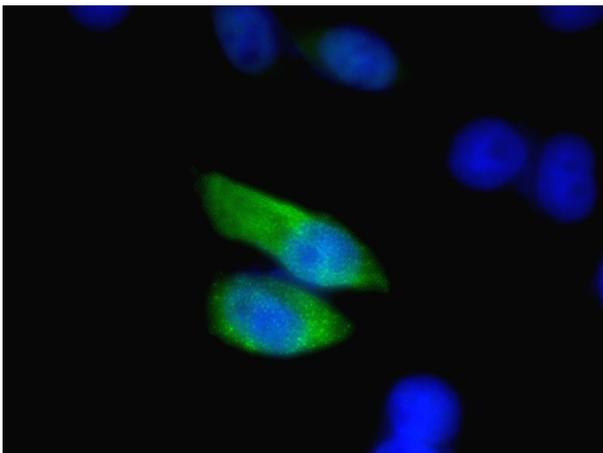
Lane 1: IP with Mouse IgG2a (isotype control) (M076-3)

Lane 2: IP with M185-3L

Immunoblotted with Anti-DDDDK-tag HRP-DirecT (PM020-7)

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) Add 200 µL of the primary antibody diluted with 2% fetal calf serum (FCS)/PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 min. at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 8) Wash the slide in a plenty of PBS as in the step 4).
- 9) Add 100 µL of 1:500 Alexa Fluor[®]488 conjugated anti-mouse IgG (Invitrogen; code no. A11001) diluted with PBS onto the cells. Incubate for 20 min. at room temperature. Keep out light by aluminum foil.
- 10) Wash the slide in a plenty of PBS as in the step 4).
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Counter stain with DAPI for 3 min. at room temperature.
- 13) Wash the slide in a plenty of PBS as in the step 4).
- 14) Promptly add mounting medium onto the slide, then put a cover slip on it.



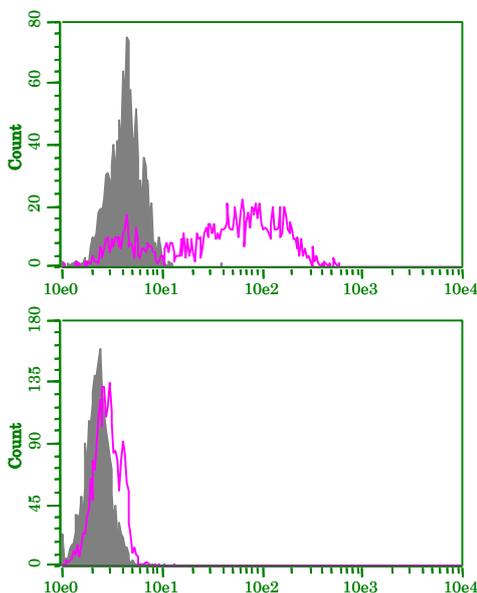
Immunocytochemical detection of DDDDK-tagged protein X in HeLa

Green: M185-3L

Blue: 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 (5×10^6 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 1 hr. at 4°C.
- 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) Add 40 μ L of 1:500 Alexa Fluor® 488 conjugated anti-mouse IgG (Invitrogen; code no. A11001) diluted with the washing buffer. Mix well and incubate for 20 min. at room temperature.
- 13) 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.
- 14) Resuspend the cells with 500 μ L of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of DDDDK-tagged protein X in HeLa

Open: M185-3L

Closed: Mouse IgG2a (isotype control) (M076-3)

Upper: DDDDK-tagged protein X in HeLa

Lower: Parental cell (HeLa)