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

My select sampler set

Anti-DDDDK-tag mAb

CODE No.	M185-3MS
CLONALITY	Monoclonal
CLONE	FLA-1
ISOTYPE	Mouse IgG2a κ
QUANTITY	20 µL, 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

Western blotting	0.1 µg/mL
Immunoprecipitation	2 µg/sample
Immunocytochemistry	0.1 µg/mL
Flow cytometry	0.05 µg/mL

APPLICATION-REPORTED

Chromatin Immunoprecipitation Reference 4)

REFERENCES

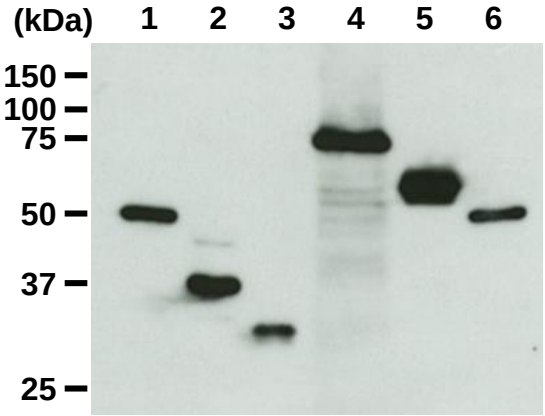
1) Nishida, K. M., *et al.*, *Nature* **555**, 260-264 (2018) [WB]
2) Tu, R., *et al.*, *Cell Death Dis.* **9**, 553 (2018) [WB, IP]
3) Yang, J., *et al.*, *PLoS Genet.* **13**, e1006975 (2017) [WB]
4) Lee, Y. K., *et al.*, *Cancer Lett.* **403**, 144-151 (2017) [WB, ChIP]
5) Song, K. H., *et al.*, *Cell 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]

For more information, please visit our web site **https://ruo.mbl.co.jp/**.

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

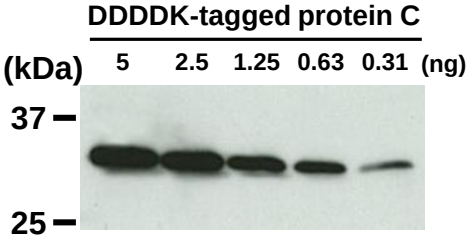
SDS-PAGE & Western blotting

1) For transfected cells: wash 1 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.).
   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 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% 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 **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).
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).
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 blotting 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

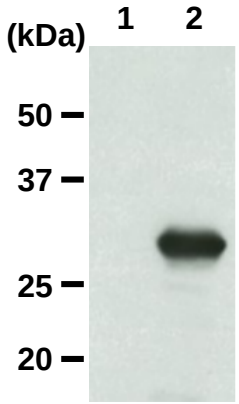
Immunoblotted with M185-3L



Western blotting analysis of DDDDK-tagged protein C
Immunoblotted with M185-3L

Immunoprecipitation

1) Mix 20 μL of 50% protein A agarose beads slurry resuspended in 300 μL 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 3)-4) twice.
6) Add 2 μg of recombinant protein in 300 μL 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 μL of Laemmli's sample buffer, boil for 2 min. and centrifuge.
9) Load 10 μL 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/cm^2 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.
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).
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.

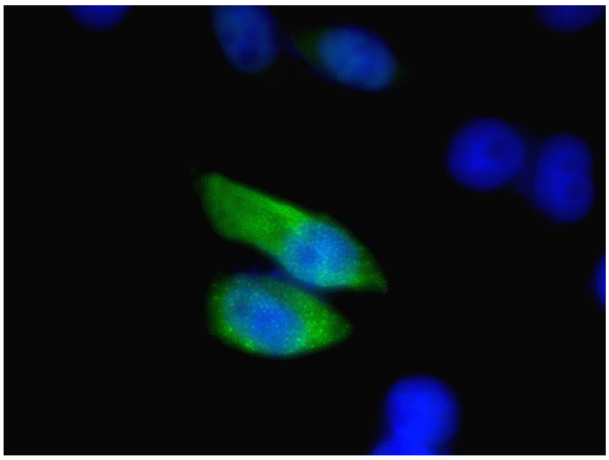


Immunoprecipitation of DDDDK-tagged GFP
Lane 1: IP with Mouse IgG2a (isotype control) (code; M076-3)
Lane 2: IP with Anti-DDDDK-tag mAb (code; M185-3L)

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

Immunocytochemistry

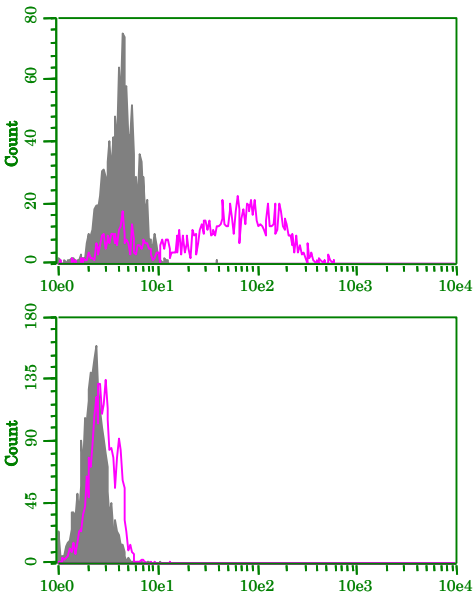
1) Spread the cells in the nutrient condition on a glass slide, then incubate in a CO_2 incubator overnight.
2) Remove the culture supernatant by careful aspiration.
3) Fix the cells by immersing the slide in 4% paraformaldehyde (PFA)/PBS for 10 min. at room temperature (20~25°C).
4) Prepare a wash container 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 (Thermo Fisher Scientific; code no. A-11001) 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: Anti-DDDDK-tag mAb (code; 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 µL 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 twice with 1 mL of washing buffer.
5) Add 200 µL 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 once with 1 mL of washing buffer.
7) Resuspend the cells with washing buffer (5 x 10^6 cells/mL).
8) Add 100 µL 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 µL 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 µL 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 µL of 1:500 Alexa Fluor® 488 conjugated anti-mouse IgG (Thermo Fisher Scientific; code no. A-11001) 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 µL of the washing buffer and analyze by a flow cytometer.



Flow cytometric detection of DDDDK-tagged protein X in HeLa

Open: Anti-DDDDK-tag mAb (code; M185-3L)
Closed: Mouse IgG2a (isotype control) (code; M076-3)

Upper: DDDDK-tagged protein X in HeLa
Lower: Parental cell (HeLa)