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

POLYCLONAL ANTIBODY

Anti-DDDDK-tag pAb

Code No.	Quantity	Form
PM020	100 µL	Affinity Purified

BACKGROUND: Epitope tagging is a powerful and versatile strategy for detecting and purifying proteins expressed by cloned genes. Short sequences encoding the epitope tag are cloned in-frame with target DNA to produce fusion proteins containing the epitope tag peptide. Due to their small size, epitope tags do not affect the tagged protein's biochemical properties. Anti-epitope tag antibodies can serve as universal purification or detection reagents for any tag-containing protein. The DDDDK epitope tag peptide sequence (DYKDDDDK) was first derived from the 11-amino-acid leader peptide of the *gene-10* product from bacteriophage T7. The DDDDK peptide has been widely used as a multi-purpose tag, and anti-DDDDK antibodies are optimally suited for identifying, detecting, purifying, and monitoring the expression levels of recombinant DDDDK fusion proteins.

SOURCE: This antibody was purified from rabbit serum using affinity column. The rabbit was immunized with KLH conjugated DYKDDDDK peptide.

FORMULATION: 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with N-terminal, Internal and C-terminal DDDDK-tagged protein on Western blotting and Immunoprecipitation.

APPLICATIONS:

Western blotting; 1:1,000 for a chemiluminescence detection system
Immunoprecipitation; 5 µL/sample
Immunohistochemistry; Not tested
Immunocytochemistry; 1:1,000
Flow cytometry; Not tested

Detailed procedures are provided in **PROTOCOLS**.

INTENDED USE:

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

REFERENCES:

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3) Kohno, T., *et al*., *J. Neurosci*. **35**, 4776-4787 (2015) [WB]
4) Murata, H., *et al*., *Mol. Biol. Cell* **24**, 2772-2784 (2013) [IC]
5) Kitagawa, M., *et al*., *PLoS One* **8**, e64826 (2013) [IC]
6) Itsumura, N., *et al*., *PLoS One* **8**, e64045 (2013) [WB]
7) Sugiyama, T., *et al*., *Nucleic Acids Res.* **41**, 6674-6686 (2013) [IP]
8) Kurio, H., *et al*., *Biol. Reprod*. **85**, 924-933 (2011) [IC]
9) Fukunaka, *et al*., *J. Biol. Chem.* **286,** 16363-16373 (2011) [IC]
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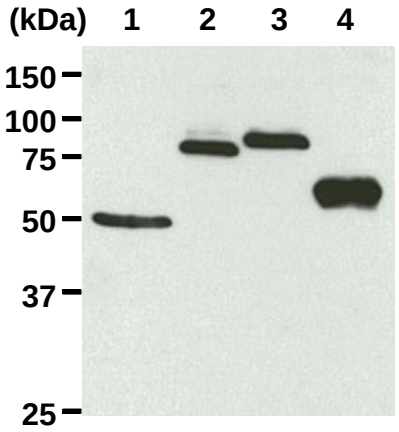
11) Takahashi, S., *J. Cell Sci.* **122**, 985-994 (2009) [WB]



Western blot analysis of DDDDK-tagged proteins using PM020

Lane 1: N-terminal DDDDK-tagged protein
Lane 2 and 3: Internal DDDDK-tagged protein/293T
Lane 4: C-terminal DDDDK-tagged protein/293T

PROTOCOLS:

SDS-PAGE & Western Blotting

1) Mix the sample with equal volume of Laemmli's sample buffer.
2) Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
3) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour 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 hour at room temperature, or overnight at 4°C.
5) Incubate the membrane for 1 hour at room temperature with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)

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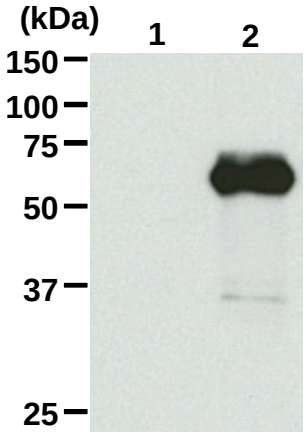
e-mail support@mbl.co.jp, TEL 052-238-1904

6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
7) Incubate the membrane with 1:10,000 of Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
8) Wash the membrane with PBS-T (5 minutes x 6 times).
9) Wipe excess buffer off the membrane, and incubate the membrane with an appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with a paper towel, and seal it in plastic wrap.
10) Expose the membrane onto an X-ray film in a dark room for 3 minutes. Develop the film under usual settings. The conditions for exposure and development may vary.



Immunoprecipitation of C-terminal DDDDK-tagged protein

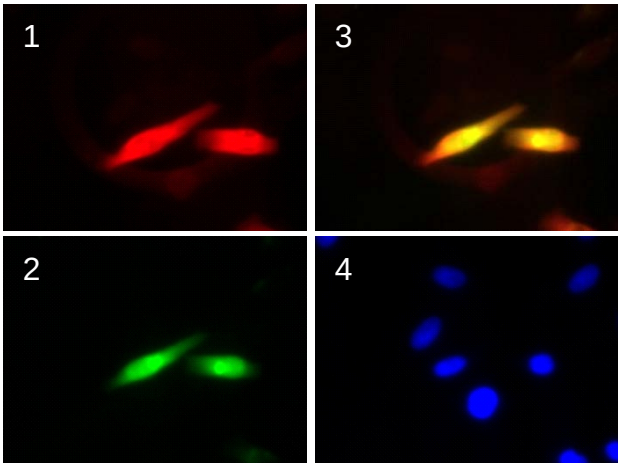
Lane 1: IP with Normal Rabbit IgG (code: PM035)
Lane 2: IP with PM020
Immunoblotted with anti-DDDDK-tag HRP-DirecT (code: PM020-7) containing Clear Back for IP Western (code: MTG-002)

Immunoprecipitation

1) Add the antibody at the amount as suggested in **APPLICATIONS** to the 5 μg of purified protein and add 200 μL of IP buffer [10 mM Tris-HCl (pH8.0), 500 mM NaCl, 0.1% NP-40]. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
2) Add 20 μL of 50% protein A agarose beads resuspended in the IP buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
3) Wash the beads 3-5 times with ice-cold IP buffer (centrifuge the tube at 2,500 x g for 10 seconds).
4) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μL/lane for SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of transfectant cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in PBS containing 4% Paraformaldehyde (PFA) for 10 minutes on ice.
3) Immerse the slide in PBS containing 0.1% Triton X-100 for 10 minutes at room temperature.
4) Add the primary antibody diluted with blocking buffer (TBS containing 5% BSA and 0.1% Tween-20) as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
5) Wash the glass slide 4 times for 5 minutes each with washing buffer (TBS containing 0.1% Tween-20).
6) Add FITC-conjugated anti-rabbit IgG antibody diluted with blocking buffer onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
7) Wash the glass slide 4 times with washing buffer for 5 minutes each.
8) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
9) Promptly add mounting medium onto the slide, then put a cover slip on it.



Immunocytochemical detection of DDDDK-tagged GFP in HeLa

1) anti-DDDDK-tag (PM020)
2) GFP own fluorescence
3) Merge, 1) and 2)
4) DAPI counter staining

RELATED PRODUCTS

Antibodies

M048-3	Anti-GFP mAb (1E4)
D153-3	Anti-GFP mAb (RQ2)
D153-6	Anti-GFP mAb-Biotin (RQ2)
D153-8	Anti-GFP mAb-Agarose (RQ2)
598	Anti-GFP pAb (polyclonal)
598-7	Anti-GFP pAb-HRP-DirecT (polyclonal)
PM073	Anti-Renilla GFP pAb (polyclonal)
M208-3	Anti-RFP mAb Cocktail (1G9, 3G5)
M155-3	Anti-RFP mAb (8D6)
M165-3	Anti-RFP mAb (3G5)
M165-8	Anti-RFP mAb-Agarose (3G5)
M204-3	Anti-RFP mAb (1G9)
M204-7	Anti-RFP mAb-HRP-DirecT (1G9)
PM005	Anti-RFP pAb (polyclonal)

PM005-7	Anti-RFP pAb-HRP-DirecT (polyclonal)
M180-3	Anti-HA-tag mAb (TANA2) (200 µL)
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)
M180-6	Anti-HA-tag mAb-Biotin (TANA2)
M180-7	Anti-HA-tag mAb-HRP-DirecT (TANA2)
561	Anti-HA-tag pAb (polyclonal) (0.1 mL)
561-A48	Anti-HA-tag pAb-Alexa Fluor® 488 (polyclonal)
561-7	Anti-HA-tag pAb-HRP-DirecT (polyclonal)
561-8	Anti-HA-tag pAb-Agarose (polyclonal)
M132-3	Anti-HA-tag mAb (5D8)
M185-3L	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)
M185-7	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)
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-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)
M047-7	Anti-Myc-tag mAb-HRP-DirecT (PL14)
M047-8	Anti-Myc-tag mAb-Agarose (PL14)
562	Anti-Myc-tag pAb (polyclonal) (0.1 mL)
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-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)
D291-8	Anti-His-tag mAb-Agarose (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)
M215-3	Anti-V5-tag mAb (OZA3)
M215-7	Anti-V5-tag mAb-HRP-DirecT (OZA3)
PM003	Anti-V5-tag pAb (polyclonal)
PM003-7	Anti-V5-tag pAb-HRP-DirecT (polyclonal)
PM003-8	Anti-V5-tag pAb-Agarose (polyclonal)
PM021	Anti-S-tag pAb (polyclonal)
PM070	Anti-E-tag pAb (polyclonal)
PM022	Anti-T7-tag pAb (polyclonal)
563	Anti-VSV-G-tag pAb (polyclonal)
M071-3	Anti-GST-tag mAb (3B2)
M209-3	Anti-GST-tag mAb (GT5)
PM022	Anti-GST-tag pAb (polyclonal)
M095-3	Anti-Luciferase mAb (2D4)
PM016	Anti-Luciferase pAb (polyclonal)
PM047	Anti-Renilla Luciferase pAb (polyclonal)
M094-3	Anti-β-galactosidase mAb (5A3)
PM049	Anti-β-galactosidase pAb (polyclonal)
M091-3	Anti-MBP (Maltose Binding Protein) mAb (1G12)
M013-3	Anti-Thioredoxin (Trx-tag) mAb (2C9)
PM015	Anti-CBD (Chitin Binding Domain) pAb (polyclonal)
PM071	Anti-Calmodulin Binding Protein-tag pAb (polyclonal)
M211-3	Anti-Strep-tag II mAb (4F1)
M214-3	Anti-mini-AID-tag mAb (1E4)

Smart-IP series

3190	Magnetic Rack
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)
M047-11	Anti-Myc-tag mAb-Magnetic beads (PL14)
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 (2E12)
M076-11	Mouse IgG2a (isotype control)-Magnetic beads (6H3)
M077-11	Mouse IgG2b (isotype control)-Magnetic beads (3D12)
M081-11	Rat IgG2a (isotype control)-Magnetic beads (2H3)
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)
M047-10	Anti-Myc-tag mAb-Magnetic Agarose (PL14)
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

3320	HA-tagged Protein PURIFICATION KIT
3321	HA-tagged Protein PURIFICATION GEL (1 mL)
3320-205	HA-tag peptide (2 mg x 5)
3325	DDDDK-tagged Protein PURIFICATION KIT
3326	DDDDK-tagged Protein PURIFICATION GEL (1 mL gel, 5 mg peptide)
3328	DDDDK-tagged Protein PURIFICATION GEL (5 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)
3300-205	c-Myc tag peptide (EQKLISEEDL) (1 mg x 5)
3306K	c-Myc-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)
3310-205	His-tag peptide (2 mg x 5)
3317	V5-tagged Protein PURIFICATION KIT Ver.2
3318	V5-tagged Protein PURIFICATION GEL Ver.2 (1 mL)
3315-205	V5-tag peptide (2 mg x 5)

Other related antibodies and kits are also available.
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