

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

Immunoprecipitation; 5 µL/sample

Immunohistochemistry; Not tested*

Immunocytochemistry; 1:1,000

Flow cytometry; Not tested

Immunofluorescence; Not tested*

*It is reported. Please visit our website at <https://ruo.mbl.co.jp/>.

Detailed procedures are provided in **PROTOCOLS**.

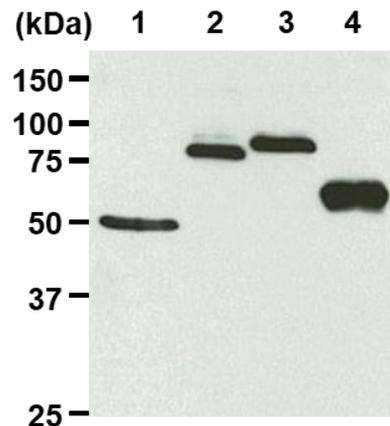
INTENDED USE:

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

REFERENCES:

Please visit our website at <https://ruo.mbl.co.jp/>.

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



Western blotting analysis of DDDDK-tagged proteins using PM020

Lane 1: N-terminal DDDDK-tagged protein

Lane 2 and 3: Internal DDDDK-tagged protein/293T

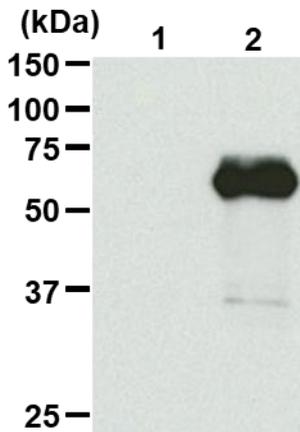
Lane 4: C-terminal DDDDK-tagged protein/293T

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/cm² for 1 hour 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) 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 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS**. (The concentration of antibody will depend on the conditions.)
- 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 pAb-HRP-Direct (code: PM020-7)

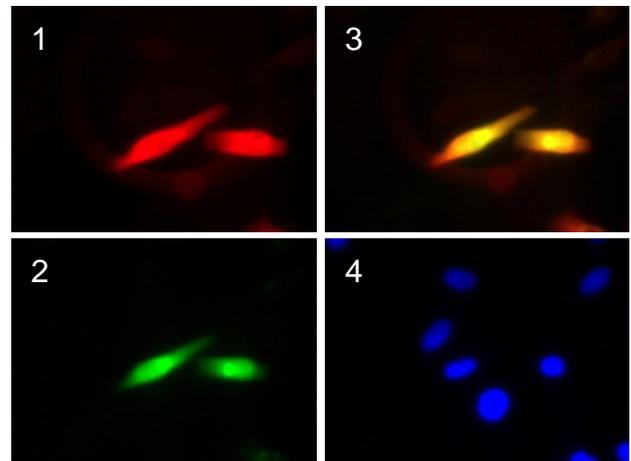
Immunoprecipitation

- 1) Add the antibody at the amount as suggested in **APPLICATIONS** to the 5 μ g of purified protein and add 200 μ L of IP buffer [10 mM Tris-HCl (pH 8.0), 500 mM NaCl, 0.1% NP-40]. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
- 2) Add 20 μ L 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) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant using a pipettor without disturbing the beads.
- 4) Resuspend the beads with ice-cold IP buffer.
- 5) Centrifuge the tube at 2,500 x g for 10 seconds, and carefully discard the supernatant.
- 6) Repeat steps 4)-5) 3-5 times
- 7) Resuspend the beads in 20 μ L of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μ L/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 CO₂ 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 pAb (PM020)
- 2) GFP own fluorescence
- 3) Merge, 1) and 2)
- 4) DAPI counter staining

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