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My select sampler set

Anti-DDDDK-tag pAb

Code No.	Quantity	Form
PM020MS	20 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: 20 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

Detailed procedure is provided in the following **PROTOCOLS**.

INTENDED USE:
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REFERENCES:
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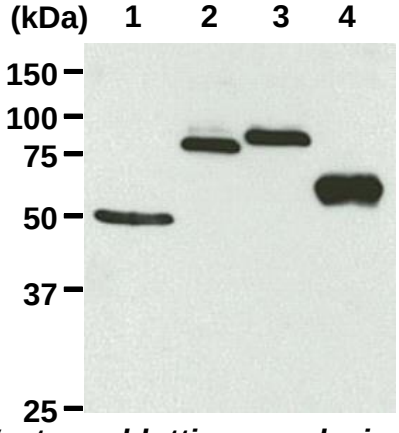


Western blotting analysis of DDDDK-tagged proteins using PM020
Lane 1: N-terminal DDDDK-tagged protein
Lane 2 and 3: Internal DDDDK-tagged protein/293T
Lane 4: C-terminal DDDDK-tagged protein/293T

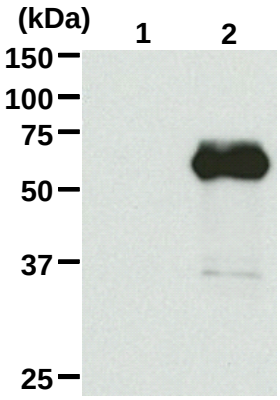
PROTOCOLS:
SDS-PAGE & Western blotting

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/cm^2 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).
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).
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

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 (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 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) 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 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**.)

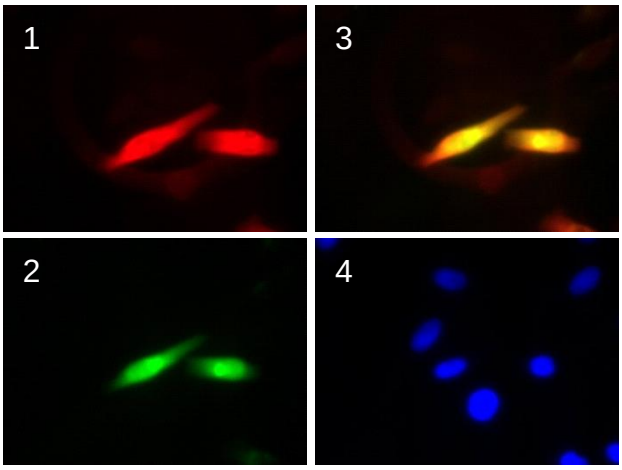


Immunoprecipitation of C-terminal DDDDK-tagged protein

Lane 1: IP with Normal Rabbit IgG (code; PM035)
Lane 2: IP with Anti-DDDDK-tag pAb (code; PM020)
Immunoblotted with Anti-DDDDK-tag pAb-HRP-DirecT (code; PM020-7)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 2 x 10^4 of transfectant cells for one slide, then incubate in a CO_2 incubator overnight)
2) Wash the cells once with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% Paraformaldehyde (PFA) for 10 minutes at room temperature.
4) Wash the glass slide twice with PBS.
5) Immerse the slide in PBS containing 0.2% Triton X-100 for 10 minutes at room temperature.
6) Wash the glass slide twice with PBS.
7) Add the primary antibody diluted with PBS containing 2% FCS as suggested in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature.
   (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) Wash the glass slide twice with PBS.
9) Add 200 µL of 1:500 Alexa Fluor® 594-conjugated Goat anti-rabbit IgG antibody (Thermo Fisher Scientific, code no. A-11012) diluted with PBS containing 2% FCS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) Add 1 drop of DAPI onto the cells and incubate for 2 minutes at room temperature. Keep out light by aluminum foil.
11) Wash the glass slide twice with PBS.
12) Wipe excess liquid off the slide but take care not to touch the cells. Never leave the cells to dry.
13) 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 (code; PM020)
2) GFP own fluorescence
3) Merge, 1) and 2)
4) DAPI counter staining

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