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MBL
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POLYCLONAL ANTIBODY

Anti-DDDDK-tag pAb-Agarose

Code No.	Quantity
PM020-8	Gel: 200 µL

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 antibody is 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: 320 µg of anti-DDDDK-tag polyclonal antibody covalently coupled to 200 µL of agarose gel and provided as a 50% gel slurry suspended in PBS containing preservative (0.09% sodium azide) for a total volume of 400 µL.

*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.

STORAGE: This antibody is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody recognizes DDDDK-tag peptide sequence (DYKDDDDK) on Immunoprecipitation.

APPLICATIONS:

Western blotting; Not tested
Immunoprecipitation; 20 µL of gel slurry
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; Not tested

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

INTENDED USE:

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REFERENCES:

1) Jing, H., *et al., Nat. Commun*. **6**, 7395 (2015) [IP]
2) Tan, L. J., *et al., Genes Cells* **17**, 173-185 (2012)
3) Fukunaka, A., *et al., J. Biol. Chem.* **286**, 16363-16373 (2011)
4) Takahashi , S., *et al., J. Cell Sci.* **122**, 985-994 (2009)
5) Maehara, T., *et al., J. Biol. Chem.* **283**, 35053-35059 (2008)

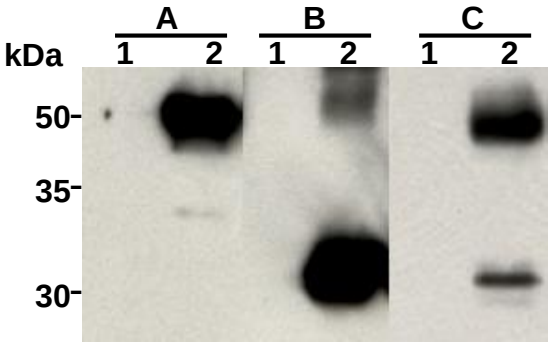
This antibody is used in these references.

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

PROTOCOL:

Immunoprecipitation

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% NP-40] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add agarose as suggest in the **APPLICATIONS** into 200 µL of cell extract. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) Resuspend the agarose in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
6) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel for electrophoresis.
7) 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 manufacture's manual for precise transfer procedure.
8) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
9) Incubate the membrane with 1 µg/mL of anti-DDDDK-tag monoclonal antibody diluted with PBS, pH 7.2 containing 1% skimmed for 1 hour at room temperature. (The concentration of antibody to be used will depend on condition.)
10) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
11) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.



Immunoprecipitation of DDDDK-tagged proteins

(A) IP from N-terminal DDDDK-tagged protein
(B) IP from Internal DDDDK-tagged protein
(C) IP from C-terminal DDDDK-tagged protein

Lane 1: IP with rabbit IgG
Lane 2: IP with PM020-8
Immunoblotted with anti-DDDDK-tag mAb

12) Wash the membrane with PBS-T (5 minutes x 3 times).
13) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
14) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

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