

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



Anti-MitoPLD (Pld6) mAb

CODE No. M207-3

CLONALITY Monoclonal
CLONE 26C46-6
ISOTYPE Mouse IgG2b κ
QUANTITY 100 μ L, 1 mg/mL

SOURCE Purified IgG from hybridoma supernatant
IMMUNOGEN KLH conjugated synthetic peptide, EFDPTKYSFPPQKHRGH (corresponding to amino acid residues 205-221 of mouse MitoPLD (Pld6))

FORMURATION 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 2-5 μ g/mL for chemiluminescence detection system
Immunoprecipitation 3 μ g/mL
Immunohistochemistry 10 μ g/mL

SPECIES CROSS REACTIVITY on WB

Species	Human	Mouse	Rat	Hamster
Tissue	Not tested	Testis	Not tested	Not tested
Reactivity		+		

Entrez Gene ID 194908 (Mouse)

REFERENCES

- 1) Nishimasu, H., *et al.*, *Nature* **491**, 284-287 (2012)
- 2) Gao, Q. and Frohman, M. A., *BMB Rep.* **45**, 7-13 (2012)
- 3) Huang, H., *et al.*, *Dev Cell.* **20**, 376-387 (2011)
- 4) Watanabe, T., *et al.*, *Dev Cell.* **20**, 364-375 (2011)
- 5) Choi, S. Y., *et al.*, *Nat Cell Biol.* **8**, 1255-1262 (2006)

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RELATED PRODUCTS

M207-3	Anti-MitoPLD (Pld6) mAb (26C46-6)
RN010MW	Anti-PIWIL1 (MIWI) mAb (2D9)
PM043	Anti-PIWIL2 (MILI) (Mouse) pAb
PM044	Anti-PIWIL2 (MILI) (Mouse) pAb
RN028P	Anti-EIF2C1 (AGO1) pAb
RN028PW	Anti-EIF2C1 (AGO1) pAb
RN003M	Anti-EIF2C2 (AGO2) (Human) mAb (1B1-E2H5)
RN005M	Anti-EIF2C2 (AGO2) mAb (2A8)
RN029PW	Anti-EIF2C2 (AGO2) pAb
RN030PW	Anti-DICER1 (Human) pAb
RN033P	Anti-TNRC6A (GW182) (Human) pAb
RN057PW	Anti-TARBP1 pAb
RN058PW	Anti-TARBP2 pAb
RN062PW	Anti-DGCR8 pAb
RN063PW	Anti-DHX9 pAb
RN065PW	Anti-KHSRP pAb
RN107PW	Anti-TARDBP (TDP-43) pAb
PD009	Anti-rck (p54) pAb
M164-3	Anti-Oct3/4 mAb (2F12)
PM048	Anti-Oct3/4 pAb
PM063	Anti-COX4 pAb
CM001-1	Anti-Cytochrome c mAb (1E4)
K0194-3	Anti-HtrA2 (Omi) (Human) mAb (18-1-83)
M202-3	Anti-Np95 (Uhrf1) mAb (R32-44)
D289-3	Anti-Np95 (Uhrf1) (Mouse) mAb (Th-10a)
5270-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit
5275-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit 2
RN1005	RIP-Assay Kit for <i>microRNA</i>
RN1011	RiboTrap Kit

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SDS-PAGE & Western blotting

1) Prepare the samples described as below:

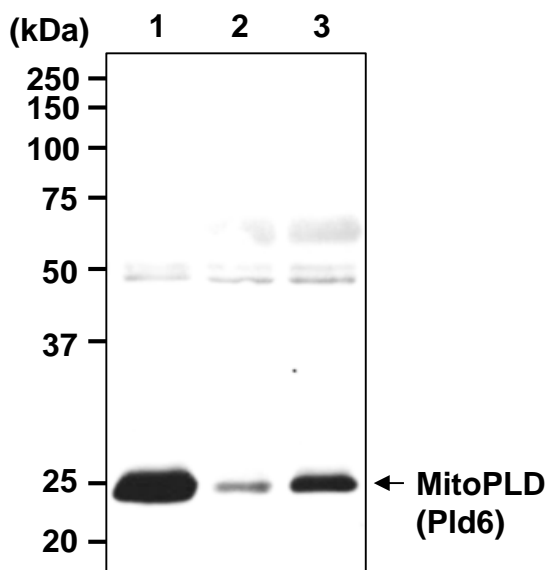
[Whole lysate]

- a) Homogenize mouse testis in 1 mL of Extraction buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA) using dounce homogenizer, then sonicate for 30 sec.
- b) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube (whole lysate).

[Cytoplasmic and mitochondrial fraction]

- a) Homogenize mouse testis in 1 mL of Isotonic Buffer (10 mM HEPES, 0.3M Mannitol, 0.1% BSA) using dounce homogenizer.
 - b) Add Digitonin solution (final concentration: 1 mM) and incubate the samples on ice for 5min.
 - c) Centrifuge the tube at 8,500 x g for 5 min. at 4°C and transfer the supernatant to another tube (cytoplasmic fraction).
 - d) Resuspend the pellet with 200 µL of Sonication buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 0.5% Tween20), then sonicate the sample (20 sec. x 3 times).
 - e) Centrifuge the tube at 10,000 x g for 5 min. at 4°C and transfer the supernatant to another tube (mitochondrial fraction).
- 2) Mix each sample with 2 x Laemmli's sample buffer, then boil for 5 min.
 - 3) Load 40 µg of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
 - 4) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
 - 5) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
 - 6) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
 - 7) Incubate the membrane with primary antibody diluted with PBS (pH 7.2) containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
 - 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
 - 9) 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 hr. at room temperature.
 - 10) Wash the membrane with PBS-T (5 min. x 3 times).
 - 11) Wipe excess buffer on the membrane, then incubate it with ECLTM WesternBlotting Detection Reagents (GE Healthcare; code no. RPN2106) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
 - 12) Expose to an X-ray film in a dark room for 10 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Mouse testis)



Western blot analysis of MitoPLD (Pld6) from mouse testis

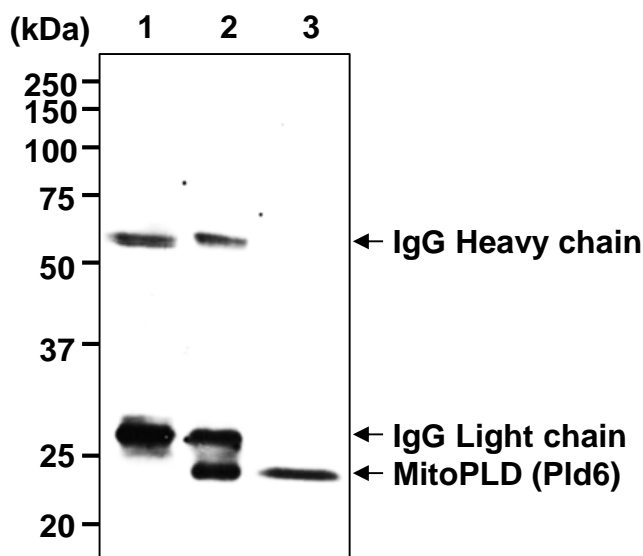
Lane 1: Mitochondrial fraction
Lane 2: Cytoplasmic fraction
Lane 3: Whole lysate

Immunoblotted with Anti-MtoPLD (Pld6) mAb (M207-3)

Immunoprecipitation

- 1) Homogenize mouse testis in 1 mL of Extraction buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA) using dounce homogenizer, then sonicate for 30 sec.
- 2) Centrifuge the tube at 12,000 x g for 5 min. at 4°C and transfer the supernatant to another tube.
- 3) Mix 30 µL of 50% protein G agarose beads slurry resuspended in 200 µL of IP buffer (50 mM Tris-HCl pH 7.4, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA) with primary antibody as suggested in the **APPLICATIONS**. Incubate with gentle agitation for 1 hr. at 4°C.
- 4) Wash the beads 3 times with 1 mL of PBS-T (0.05% Tween-20 in PBS).
- 5) Add 100 µL of tissue lysate (prepared sample from step 2)) and 200 µL of IP buffer into the tube. Incubate with gentle agitation for 1 hr. at 4°C.
- 6) Wash the beads 4 times with 1 mL of PBS-T.
- 7) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 5 min. and centrifuge.
- 8) Load 10 µL of the sample per lane in a 1-mm-thick SDS-polyacrylamide gel (12.5% acrylamide) for electrophoresis.
- 9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hr. 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.
- 10) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) overnight at 4°C.
- 11) Wash the membrane with PBS-T (5 min. x 3 times).
- 12) Incubate the membrane with 5 µg/mL of anti-MitoPLD (Pld6) mAb (MBL; code no. M207-3) 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 (5 min. x 3 times).
- 14) 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 hr. at room temperature.
- 15) Wash the membrane with PBS-T (5 min. x 3 times)
- 16) Wipe excess buffer on the membrane, then incubate it with ECLTM WesternBlotting Detection Reagents (GE Healthcare; code no. RPN2106) for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 17) Expose to an X-ray film in a dark room for 5 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Immunoprecipitation; Mouse testis)



Immunoprecipitation of MitoPLD (Pld6) from mouse testis

Lane 1: Mouse IgG2b (MBL; code no. M077-3)

Lane 2: Anti-MitoPLD (Pld6) mAb (M207-3)

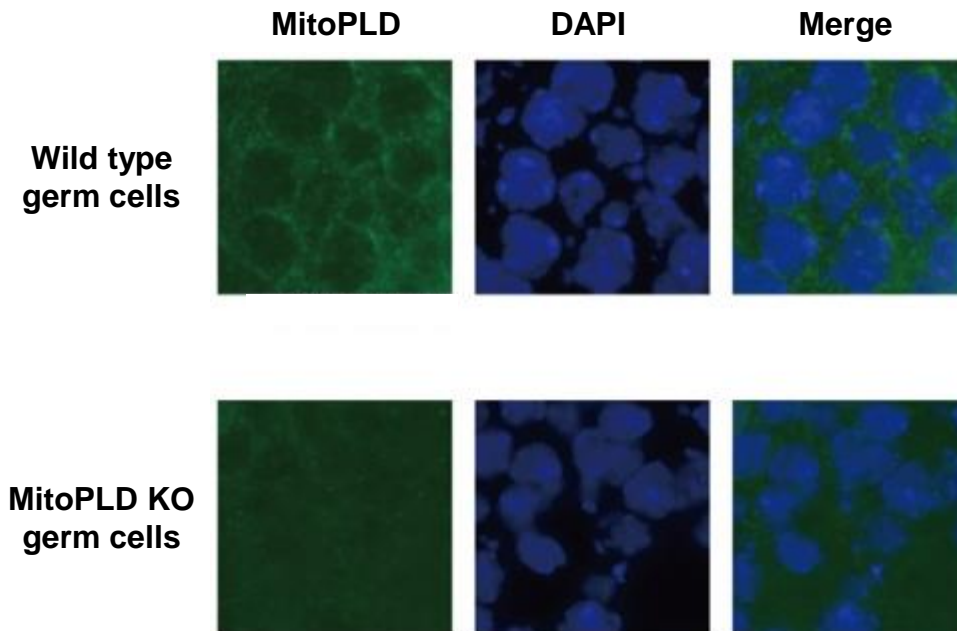
Lane 3: Whole lysate (40 µg)

Immunoblotted with Anti-MtoPLD (Pld6) mAb (M207-3)

Immunohistochemistry (frozen section)

- 1) Fix frozen section in 4% paraformaldehyde (PFA)/PBS for 10 min. at 4°C.
- 2) Wash the slides with PBS.
- 3) Immerse the slides in permeabilization buffer (0.5% BSA, 0.5% Triton X-100/PBS) for 15 min. at room temperature.
- 4) Briefly wash the slides with PBS.
- 5) Immerse the slides in blocking buffer (0.2% BSA, 0.1% Tween20, 0.1% Gelatin/PBS) for 30 min. at room temperature.
- 6) Incubate the slides with the primary antibody diluted with blocking buffer as suggested in the **APPLICATIONS** for overnight at 4°C. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 7) Wash 3 times for 5 min. each with PBS.
- 8) Incubate the slides with 2 µg/mL of Alexa Fluor® 488 Goat Anti-mouse IgG (Invitrogen; code no. A11017) diluted with blocking buffer for 1 hr. at room temperature in dark chamber.
- 9) Wash 3 times for 5 min. each with PBS.
- 10) Wipe excess liquid from the slide. Add VECTASHIELD Mounting Medium with DAPI (Vector Laboratories; code no. H-1200) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Postnatal day 20 mouse testis)



Immunohistochemical detection of MitoPLD (Pld6)

Data were kindly provided by Yuka Kabayama, M.M.S., and Prof. Hiroyuki Sasaki, M.D., Ph.D. (Division of Epigenomics and Development, Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University)