

## Anti-MitoPLD (Pld6) mAb

<b>CODE No.</b>	M207-3
<b>CLONALITY</b>	Monoclonal
<b>CLONE</b>	26C46-6
<b>ISOTYPE</b>	Mouse IgG2b $\kappa$
<b>QUANTITY</b>	100 $\mu$ L, 1 mg/mL
<b>SOURCE</b>	Purified IgG from hybridoma supernatant
<b>IMMUNOGEN</b>	KLH conjugated synthetic peptide, EFDPTKYSFFPQKHRGH (corresponding to amino acid residues 205-221 of mouse MitoPLD (Pld6))
<b>FORMULATION</b>	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
<b>STORAGE</b>	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

### APPLICATIONS-CONFIRMED

<u>Western blotting</u>	2-5 $\mu$ g/mL
<u>Immunoprecipitation</u>	3 $\mu$ g/mL
<u>Immunohistochemistry</u>	10 $\mu$ 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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The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

## **SDS-PAGE & Western blotting**

1) Prepare the samples described as below:

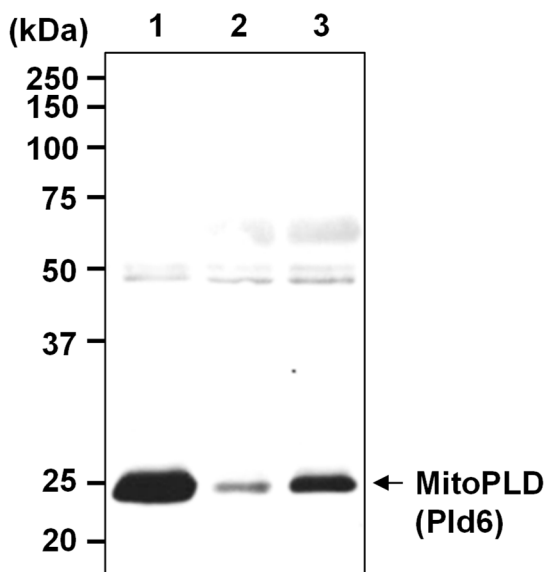
[Whole lysate]

- 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/cm<sup>2</sup> 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 ECL<sup>TM</sup> 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**

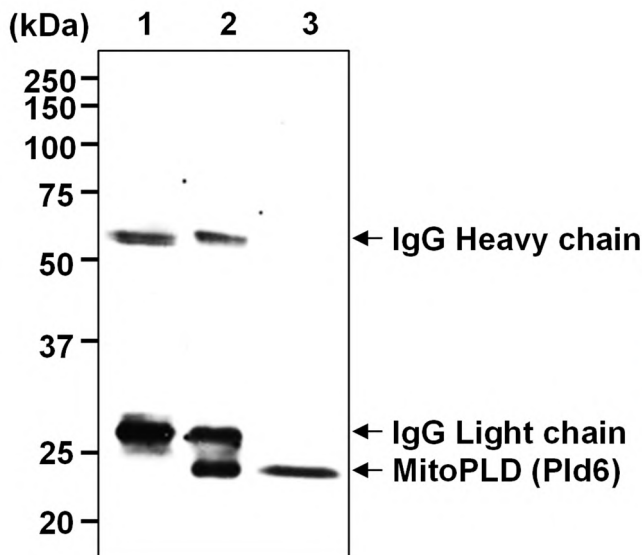
Lane 1: Mitochondrial fraction  
Lane 2: Cytoplasmic fraction  
Lane 3: Whole lysate

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/cm<sup>2</sup> 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 ECL<sup>TM</sup> 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)

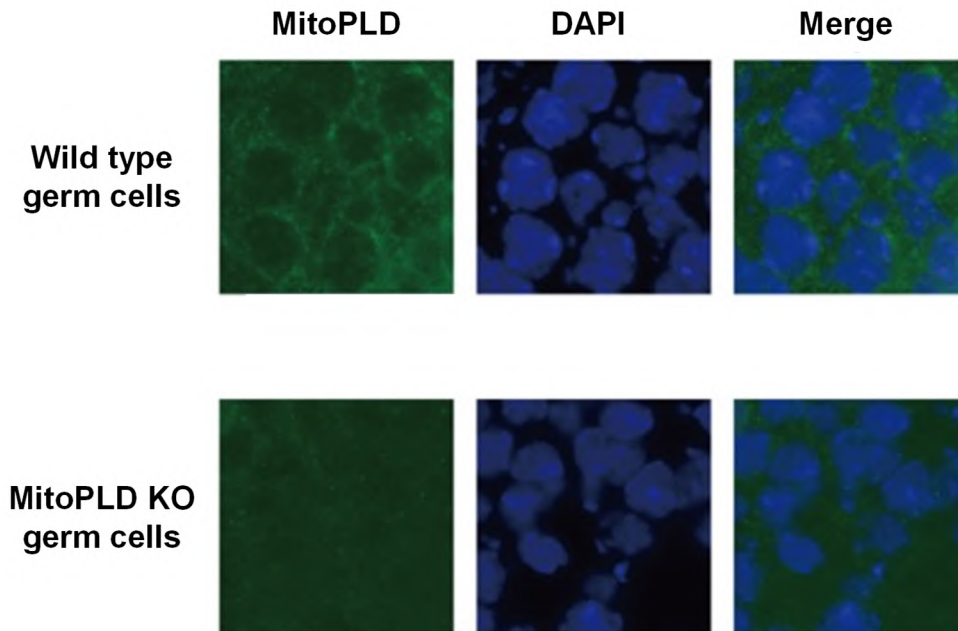
Lane 3: Whole lysate (40 µg)

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)