**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 µL, 1 mg/mL |
| **SOURCE** | Purified IgG from hybridoma supernatant |
| **IMMUNOGEN** | KLH conjugated synthetic peptide, EFDPTKYSFFPQKHRGH (corresponding to amino acid residues 205-221 of mouse MitoPLD (Pld6) |
| **FORMULATION** | 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**

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|---|---|
| Western blotting | 2-5 µg/mL |
| Immunoprecipitation | 3 µg/mL |
| Immunohistochemistry | 10 µg/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.

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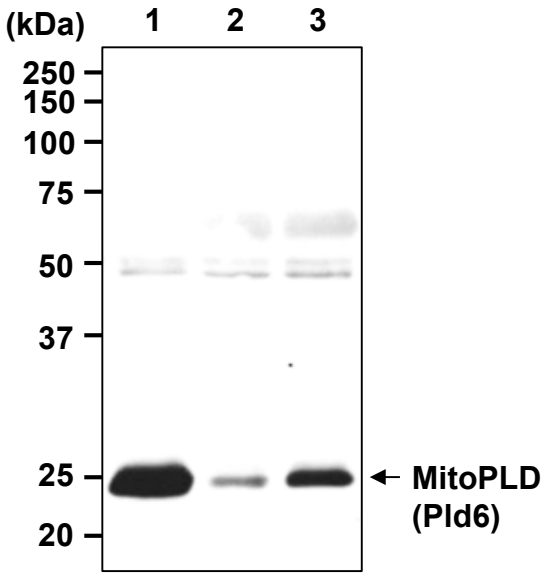
## SDS-PAGE & Western blotting

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/cm$^2$ 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™ 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)



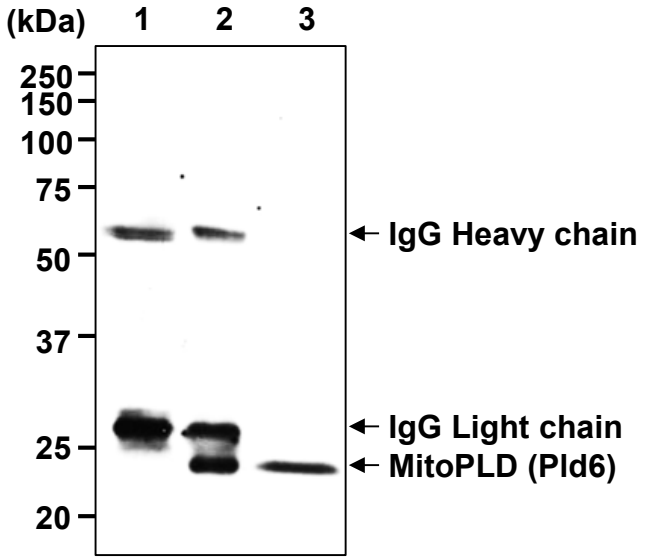

***Western blot analysis of MitoPLD (Pld6) from mouse testis***
Lane 1: Mitochondorial fraction
Lane 2: Cytoplasmic fraction
Lane 3: Whole lysate

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$^2$ 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™ 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 Immunopirecipitation; Mouse testis)




***Immunoprecipitation of MitoPLD (Pld6) from mouse testis***

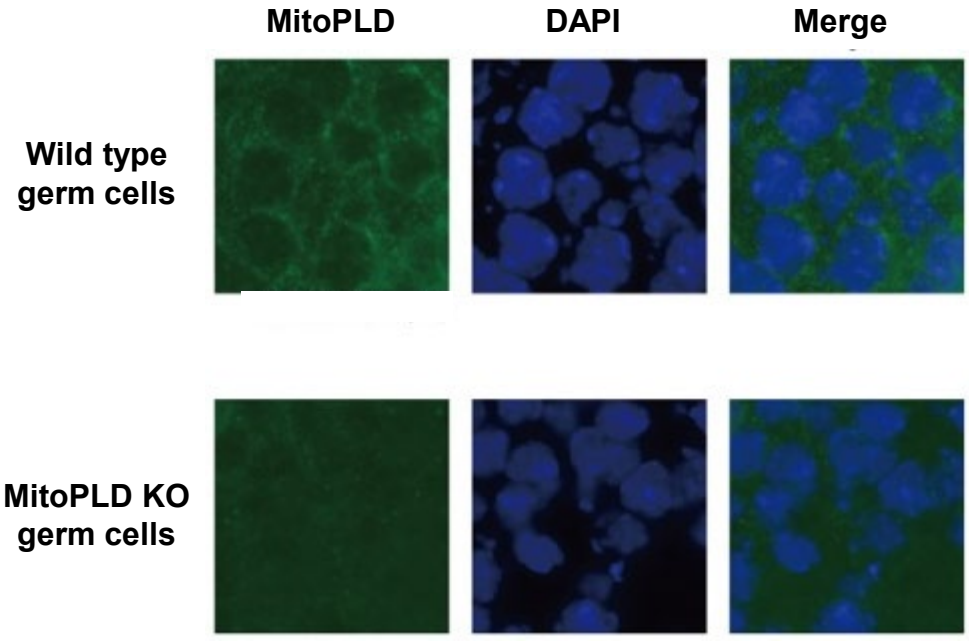
Lane 1: Mouse IgG2b (MBL; code no. M077-3)
Lane 2: Anti-MitoPLD (Pld6) mAb (M207-3)
Lane 3: Whole lysate (40 µg)

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)