

Anti-PDILT antibody, rabbit serum, KO-Validated

73-051 100 µl

Shipping and Storage: Shipped at 4°C or -20°C and store at -20°C.

Immunogen: Two synthetic peptides corresponding to the C-terminal regions of mouse PDILT, C+IRKPEEPERRKETA (550-563) and C+QPKEQPKPERKLEV (571-584), respectively, conjugated with KLH

Validation: Specificity validated with knock-out mice.

Form: Whole rabbit antiserum added with 0.1% sodium azide.

Reactivity: Mouse. Not tested in other species.

Applications:

1. Western blotting (1/1,000 dilution)
2. Immunoprecipitation (1/100)
3. Immunohistochemistry (1/100-1/500)

Background: PDILT cooperates with the testis-specific calreticulin-like chaperone, calsperin (CALR3), in the endoplasmic reticulum and plays an indispensable role in the disulfide-bond formation and folding of ADAM3. *Pdilt*(-/-) mice were male infertile because ADAM3 could not be folded properly and transported to the sperm surface without the PDILT/CALR3 complex.

Molecular mass: 67,759 with 588 amino acids. N-glycosylated

Not tested for other applications.

Database Links: [uniprot/Q9DAN1](#) Mouse PDILT, [Gene ID 71830](#) mouse *Pdilt*

Reference: This antibody was described and used in the following publication..

Tokuhiro K. et al (2012). Protein disulfide isomerase homolog PDILT is required for quality control of sperm membrane protein ADAM3 and male fertility. [Proc Natl Acad Sci U S A](#). 109:3850-5. **WB, IP, IHC**. Free access.

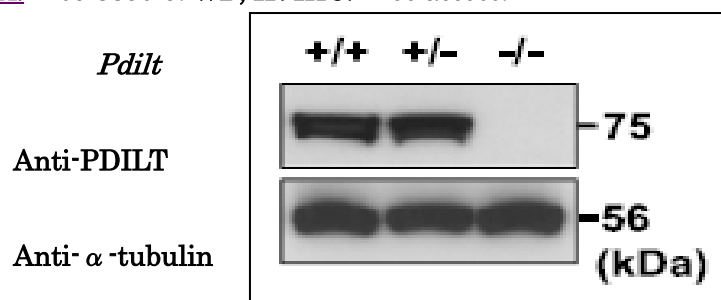


Fig. 1. Validation of specificity of the anti-PDILT antibody with knockout mice testis extracts. Samples wild-type, hybrid and knock-out were reacted with anti-PDILT antibody at 1/1,000 dilution. α -Tubulin was used as a control.

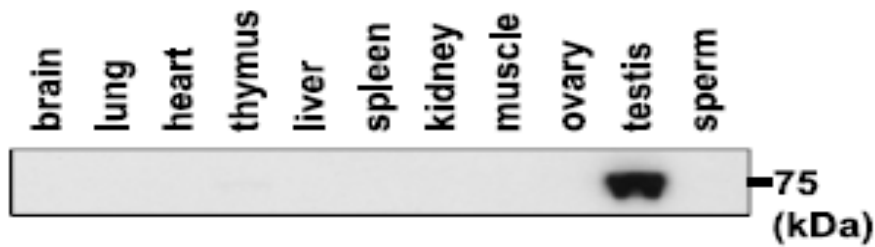


Fig.2 Western blotting analysis of PDILT expression in various tissues with anti-PDILT antibody. Proteins were extracted from various tissues with lysis buffer containing Triton X-100 and subjected to western blot analysis. Tissue proteins (30 μ g) and sperm protein (10 μ g) were reacted with anti-PDILT antibody at 1/1,000 dilution.

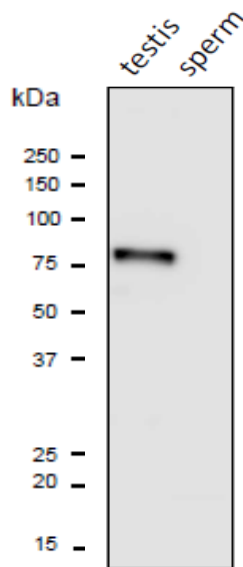


Fig.3 Western blot analysis of PDILT protein in the lysates of mouse testis and sperm with anti-PDILT antibody.

Proteins in the lysates (10 μ g) were separated on SDS-PAGE, electro-blotted to PVDF membrane and reacted with anti-PDILT antibody at 1/1,1000 dilution. Anti-rabbit IgG conjugated with HRP was used at 10,000 dilution.

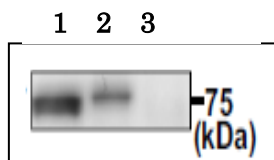
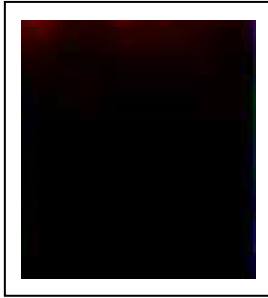


Fig.4. Immunoprecipitation of PDILT protein from mouse testis extracts. Extracts (100 μ g) of wild-type (+/+) and *Pdilt* (-/-) mouse testes were immunoprecipitated with anti-PDILT antibody at 1/100 dilution and the precipitates were analyzed by western blotting using the same antibody at 1/1,000 dilution.

1. Input.
2. Wild-type.
3. *Pdilt* (-/-)

Spermatocyte



Spermatid

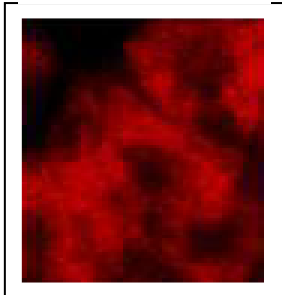


Fig.5. Immunofluorescence staining of mouse testicular sections with anti-PDILT antibody. Testes were collected from adult mice and were fixed in 4% paraformaldehyde/PBS, cryopreserved in graded 10-30% sucrose, and embedded in a TissueTek OCT compound (Sakura Finetechnical, Tokyo). Frozen sections (5 μ m) were mounted on APS-coated glass slides. After washing and blocking, the slides were reacted with anti-PDILT antibody at 1/100 dilution. As a secondary antibody, Alexa Fluor 546 conjugated anti-rabbit IgG antibody was used. PDILT was detected only in spermatid .

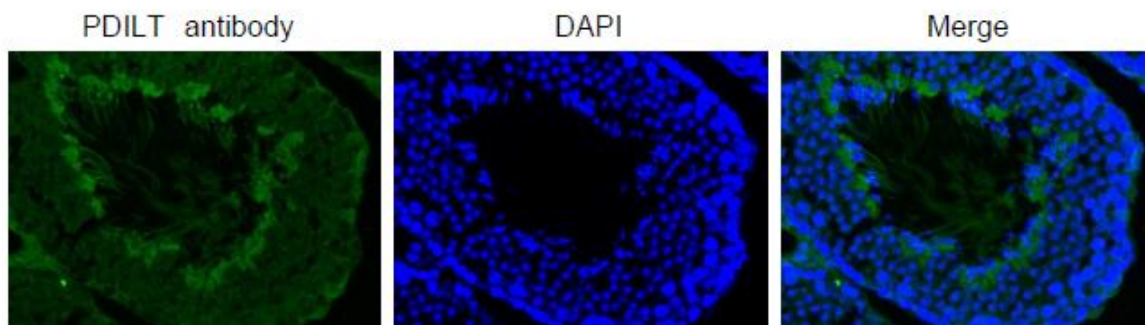


Fig.6 Immunohistochemical analysis of paraffin embedded mouse testis tissue labelling PDILT with PDILT antibody

Deparaffinization LemosolRA (#122-03991,Wako, Osaka)

Rehydration

Antigen retrieval Histo/Zyme (Cat.# k046; Diagnostic BioSystems)

Washing PBST (0.25% triton X-100/PBS-)

Blocking 10 % FBS / PBST 30 min

1st antibody 1/1,000 dilution in PBS- 4°C O/N

Washing PBS-

2nd antibody 1,000 dilution, 60 min

washing PBS- 5 min X 3

DAPI 1.0 μ g/mL DAPI in TBS 10 min