Anti-PDILT antibody, rabbit polyclonal, KO-Validated

Product code	73-713
Size	100 µg
Storage	-20℃
Concentration	1mg/ml
Buffer	PBS- with 50 % glycerol
Purity	Purified IgG fraction with protein A from rabbit antiserum (73-051).
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
Isotype	Rabbit IgG
Reactivity	Mouse. Not tested with other species.
Special notes	Validation: knock-out mouse
Application	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-glucosylated
Data Link	UniProtKB Q9DAN1 Mouse PDILT, Gene ID 71830 Mouse PDILT
Reference	This antibody was described and used in the following publication. 1. 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.
Related Products	73-051 Anti-PDILT antibody, rabbit serum
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

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Data Images: 73-713 Anti-PDILT antibody, rabbit polyclonal, KO-Validated

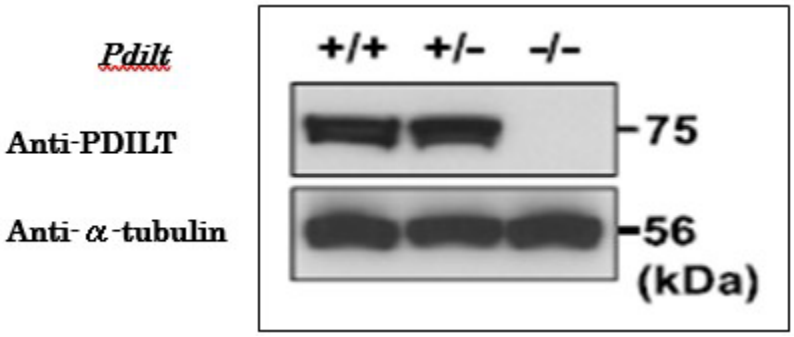


Fig. 1. Validation of specificity of the anti-PDILT antibody with knockout mice testis extracts. Samples wild-type, hybrid and knock-out were reacted with anti-PDILT antibody at 1/1,000 dilution. α-Tubulin was used as a control.

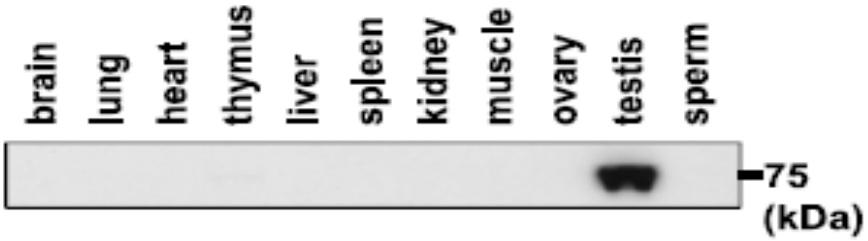


Fig.2 Western blotting analysis of PDILT expression in various tissues with anti-PDILT antibody. Proteins were extracted from various tissues with lysis buffer containing Triton X-100 and subjected to western blot analysis. Tissue proteins (30 μg) and sperm protein (10 μg) were reacted with anti-PDILT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

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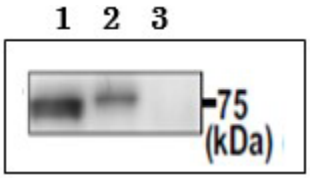


Fig.4. Immunoprecipitation of PDILT protein from mouse testis extracts. Extracts (100 μg) of wild-type (+/+) and *Pdilt* (-/-) mouse testes were immunoprecipitated with anti-PDILT antibody at 1/100 dilution and the precipitates were analyzed by western blotting using the same antibody at 1/1,000 dilution.

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

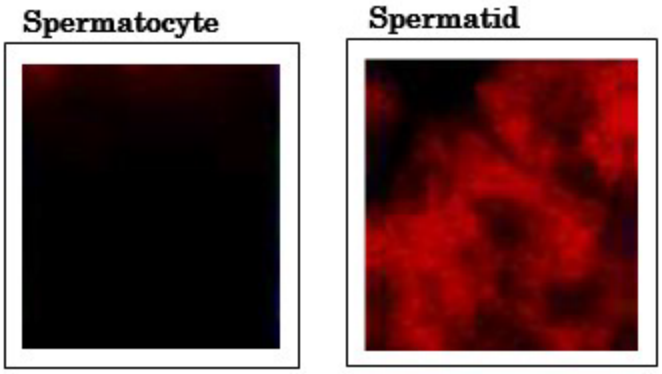


Fig.5. Immunofluorescence staining of mouse testicular sections with anti-PDILT antibody.

Testes were collected from adult mice and were fixed in 4% paraformaldehyde/PBS, cryopreserved in garaded 10-30% sucrose, and embedded in a TissueTek OCT compound (Sakura Finetechnical, Tokyo). Frozen sections (5 μm) 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.

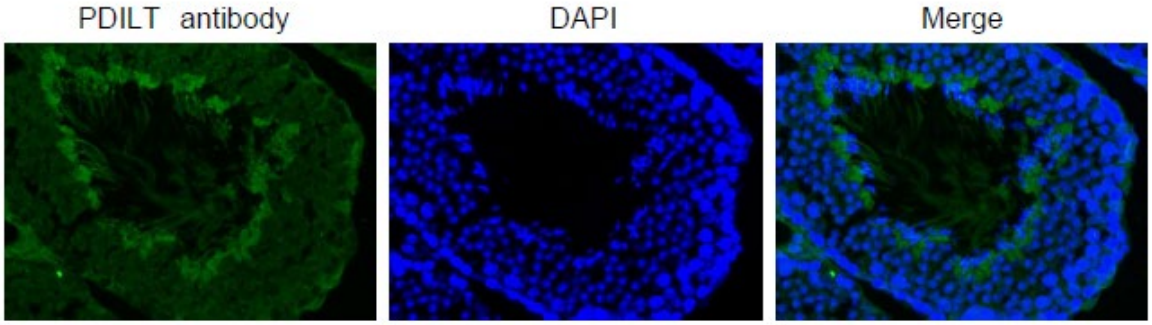


Fig.6 Immunohistochemical analysis of paraffin embedded mouse testis tissue labelling PDILT with PDILT antibody.

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

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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℃ O/N
Washing	PBS-
2nd antibody	1,000 dilution, 60 min
washing	PBS- 5 min X 3
DAPI	1.0μg/mL DAPI in TBS 10 min
Washing	PBS-
Mount	ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)

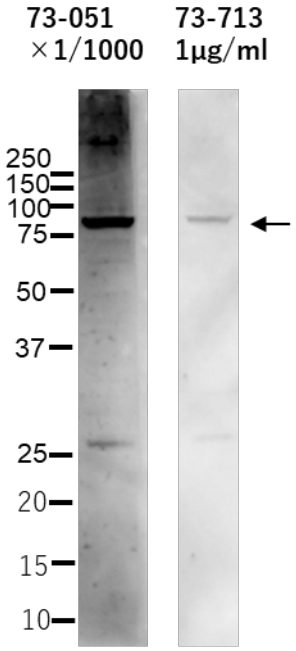


Fig.7 Western blot analysis of PDILT protein in the lysates of mouse testis with anti-PDILT antibody.

Proteins in the lysates (10 μg) 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.

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