

## Anti-PDILT antibody, rabbit polyclonal, KO-Validated

Cat. #73-051      Size: 100 µl

### 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

### Specifications:

**Reactivity:** Mouse. Not tested in other species.

**Validation:** Specificity validated with knock-out mice.

**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

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

**Storage:** Ship at 4°C and store at -20°C.

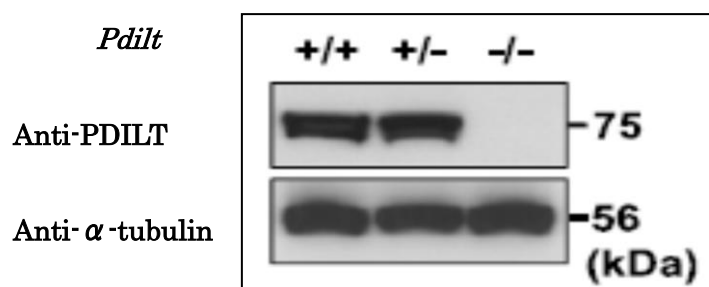
### Applications:

- Western blotting (1/1,000 dilution)
  - Immunoprecipitation (1/100)
  - Immunohistochemistry (1/100-1/500)
- Not tested for other applications.

**Database Links:** [uniprot/Q9DAN1](https://uniprot.org/Q9DAN1) Mouse PDILT, [Gene ID 71830](https://ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE71830) mouse *Pdilt*

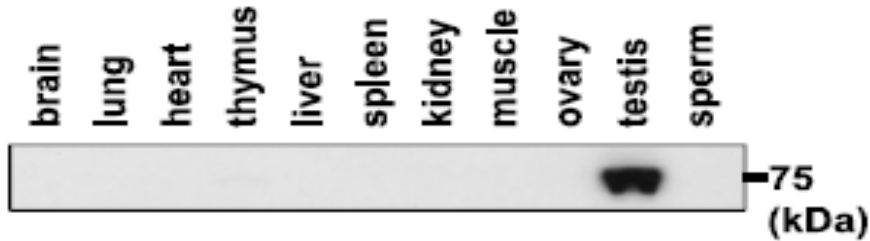
**Reference:** This antibody was described and used in the following publication.

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](https://doi.org/10.1073/pnas.1119850109). 109:3850-5. **WB, IP, IHC**. Free access.



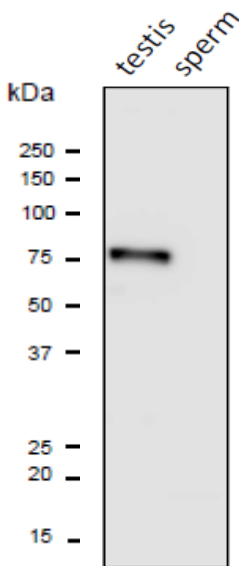
**Fig. 1. Validation of specificity of the anti-PDILT antibody with knockout mice testis extracts.**

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.



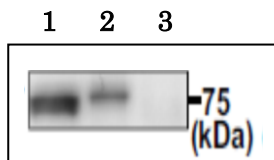
**Fig.2 Western blotting analysis of PDILT expression in various tissues with anti-PDILT antibody.**

Proteins were extracted from various tissues with lysis buffer containing Triton X-100 and subjected to western blot analysis. Tissue proteins (30 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 1/1,000 dilution. Anti-rabbit IgG conjugated with HRP was used at 10,000 dilution.

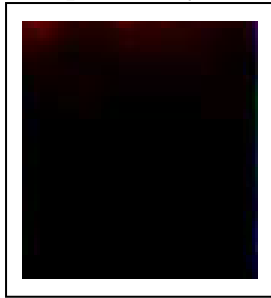


**Fig.4. Immunoprecipitation of PDILT protein from mouse testis extracts.**

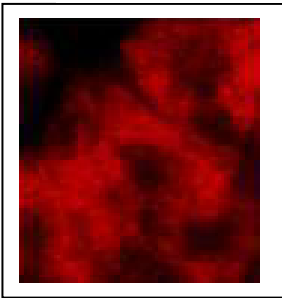
Extracts (100 µg) of wild-type (+/+) and *Pdilt* (-/-) mouse testes were immunoprecipitated with anti-PDILT antibody at 1/100 dilution and the precipitates were analyzed by western blotting using the same antibody at 1/1,000 dilution.

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

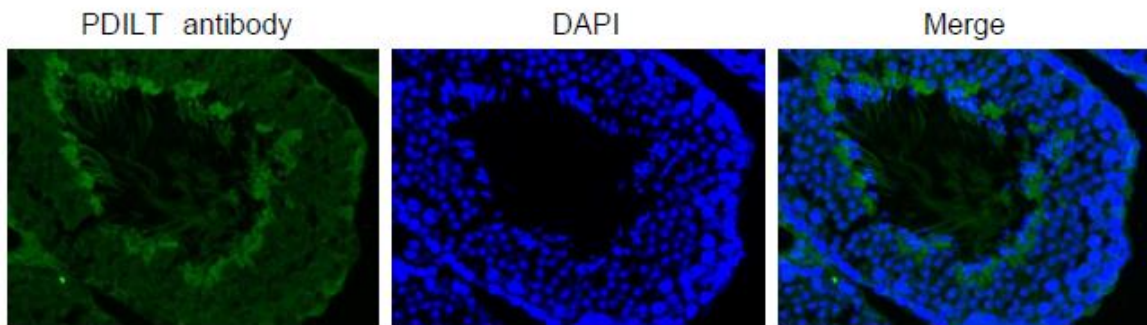
**Spermatocyte**



**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  $\mu$  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 $\mu$ g/mL DAPI in TBS		10 min
Washing	PBS-		
Mount	ImmunoSelect Antifading Mounting Medium	(SCR-38447;	