

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

Cat.#73-034, Size:100 µl

### Background:

Calmegin plays an important role in sperm fertility. Binds calcium ions. Specifically expressed during male meiotic germ cell development.

Molecular mass: 69,431 with 611 amino acids

**Key words:** Calmegin, CLGN, Spermatogenesis, Endoplasmic reticulum, Chaperon, Transmembrae, PDILT, Calcium ion binding, Binding of sperm to zona pellucida

### Specifications:

**Validation:** Specificity validated with knock-out mouse (Fig.2.)

**Reactivity:** Mouse.

**Immunogen:** C-terminal peptide of mouse Calmegin, DESPGSGDAPLKSLRKRRVRKD, conjugated with KLH

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

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

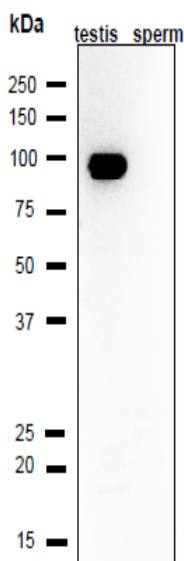
### Applications:

- Western blotting (1/1,000 dilution))
- Immunoprecipitation (1/100~1/1,000 dilution).
- Immunofluorescent staining (1/100~1/1,000 dilution)
- Immunohistochemistry (Paraffin embedded) (1/1,000 dilution)

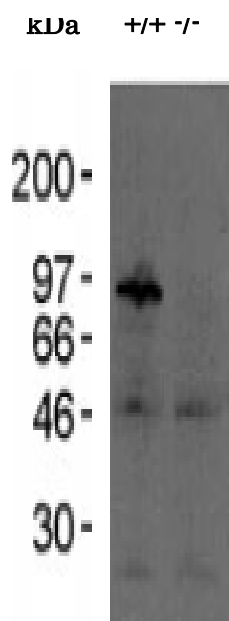
**Database Links:** [uniprot/P52194](#) mouse Calmegin, [Gene ID 12745](#) mouse Clgn

**Reference:** This antibody was described in Ref.1 and used in the following publications.

1. Ikawa M. et al. (2001) Calmegin Is Required for Fertilin  $\alpha/\beta$  Heterodimerization and Sperm Fertility. [Dev Biol.](#) 240: 254-61. **WB, IP.** Open access.
2. Ikawa M. et al. (2011) Calsperin is a testis-specific chaperone required for sperm fertility. [J Biol Chem.](#) 286: 5639-46. **WB, IP.** Open access.

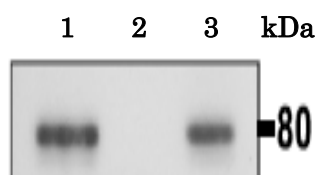


**Fig.1 Western blot analysis of Calmegin in lysates of mouse testis and sperm.** Proteins in the lysates (10 µg) were separated on SDS-PAGE (10~20% gel) and blotted to PVDF membrane. It was reacted with anti-Calmegin-antibody at 1/1,000 dilution. As the second antibody, goat anti-rabbit IgG conjugated with HRP (Abcam; ab97051) was used at 1/10,000 dilution.



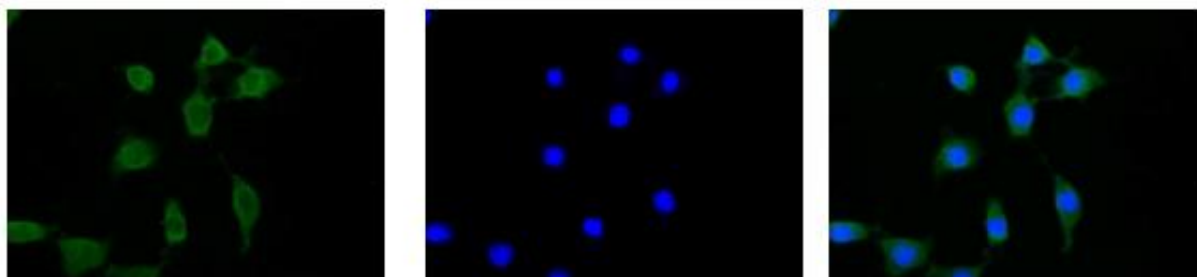
**Fig.2 Western blotting analysis of testis extracts of wild-type and knockout mice with anti-Calmegin antibody.**

20 µg of Triton X-100 extracts from mouse testes was reacted with anti-Calmegin antiserum at 1/1,000 dilution. Arrow indicates the position of intact Calmegin.



**Fig.3. Immunoprecipitation of Calmegin from mouse testis.** One mg of testis lysate was incubated with 2 µl of anti-Calmegin antiserum and 50 µl. of protein-A conjugated magnetic beads (Milenyi Biotec) and immunoprecipitated according to the protocol of supplier. The immunoprecipitated sample was analyzed by western blotting with the antibody at 1/1,000 dilution.

1. Input    2. non-immune serum    3. Anti-Calmegin antiserum



**Fig.4 Immunofluorescent staining of Calmegein in NIH3T3 cells with anti-Calmegein antibody**

Fixation of the cells in 4% paraformaldehyde overnight

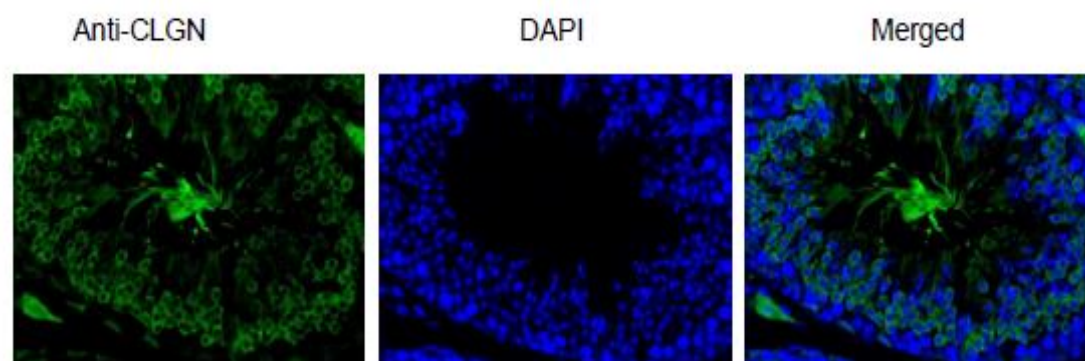
Permeabilization in 0.25% Triton X-100/PBS for 10 min

Blocking in 1.5% BSA/PBS for 30 min

1st antibodies 1/100 diluted by blocking buffer over night

2nd Goat anti rabbit IgG conjugated with Alex 488 (1:1000 dilution) for 60min

Nuclei were stained with DAPI



**Fig.5 Immunohistological staining of Calmegein in testis section with anti-Calmegein 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 Goat anti rabbit IgG conjugated with Alex 488 (1:1000 dilution) for 60min

Washing PBS- 5 min, 3 times

DAPI 1.0µg/mL DAPI in TBS 10 min (×100 stock, 0.1mg/mL)

Washing PBS-

Mount ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)