

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

73-034 100  $\mu$ l

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

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

**Reactivity:** Mouse.

### Applications:

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

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

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

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

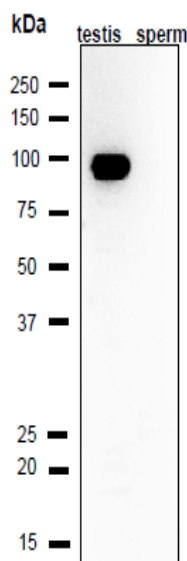
**Function:** Calmegin plays an important role in sperm fertility. Binds calcium ions. Specifically expressed during male meiotic germ cell development.

**Molecular mass:** 69,431 with 611 amino acids

**Database Links:** [uniprot/P52194](https://uniprot.org/P52194) mouse Calmegin , [Gene ID 12745](https://ncbi.nlm.nih.gov/nuccore/12745) mouse Clgn

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

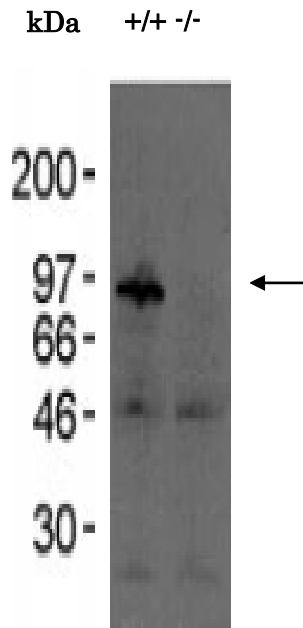
1. Ikawa M. et al. (2001) Calmegin Is Required for Fertilin  $\alpha/\beta$  Heterodimerization and Sperm Fertility. [Dev Biol.](#) 240: 254-61. **WB, IP.** Open access.
2. Ikawa M. et al. (2011) Calsperin is a testis-specific chaperone required for sperm fertility. [J Biol Chem.](#) 286: 5639-46. **WB,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



**Fig.1 Western blot analysis of Calmegin in lysates of mouse testis and sperm.**

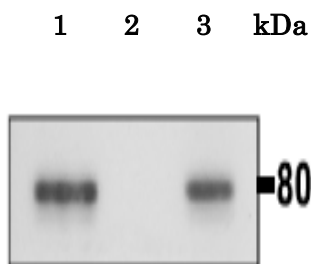
Proteins in the lysates (10  $\mu$ g) were separated on SDS-PAGE (10~20% gel) and blotted to PVDF membrane. It was reacted with anti-Calmegin-antibody at 1/1,000 dilution.

As the second antibody, goat anti-rabbit IgG conjugated with HRP (Abcam; ab97051) was used at 1/10,000 dilution.



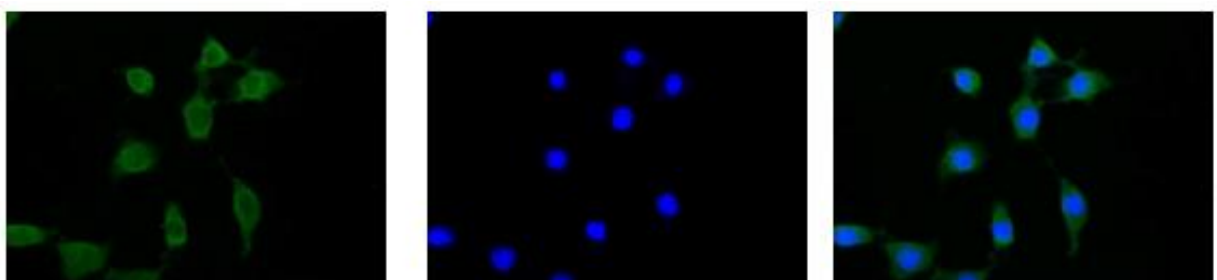
**Fig.2 Western blotting analysis of testis extracts of wild-type and knockout mice with anti-Calmegin antibody.**

20  $\mu$ g of Triton X-100 extracts from mouse testes was reacted with anti-Calmedin 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  $\mu$ l of anti-Calmegin antiserum and 50  $\mu$ l. of protein-A conjugated magnetic beads (Miltenyi 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 Calmegin in NIH3T3 cells with anti-Calmegin antibody**

Fixation of the cells in 4% paraformaldehyde over night

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

