

Anti-Calmegin antibody, rabbit serum, KO-Validated

73-034 100 µl

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

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

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

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

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)

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

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

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 α/β 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. [JBiol Chem.](#) 286: 5639-46. **WB, IP.** Open access.

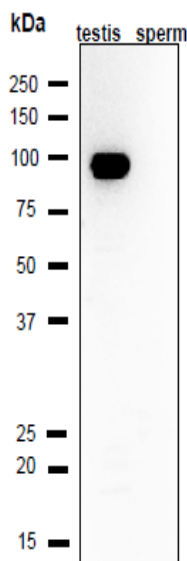


Fig.1 Western blot analysis of Calmegin in lysates of mouse testis and sperm.

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.

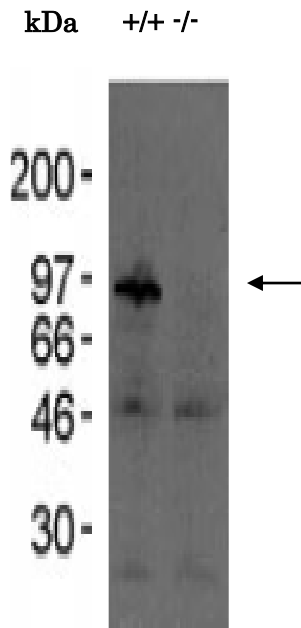


Fig.2 Western blotting analysis of testis extracts of wild-type and knockout mice with anti-Calmegin antibody.

20 μ 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.

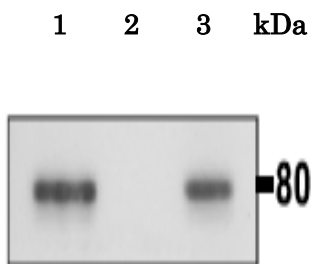


Fig.3. Immunoprecipitation of Calmegin from mouse testis. One mg of testis lysate was incubated with 2 μ l of anti-Calmegin antiserum and 50 μ l. of protein-A conjugated magnetic beads (Miltenyi Biotec) and immunoprecipitated according to the protocol of supplier. The immunoprecipitated sample was analyzed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

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

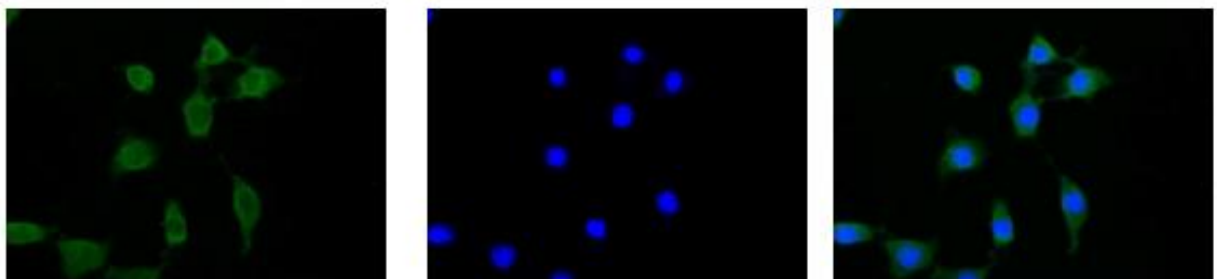


Fig.4 Immunofluorescent staining of Calmegin in NIH3T3 cells with anti-Calmegin antibody

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

