

Anti-Calmegin antibody, rabbit serum

Product code	73-034
Size	100 µl
Storage	Store 4°C for short term For long term storage store at -20°C. Aliquot to avoid repeated freezing and thawing.
Concentration	N/A
Buffer	0.1% sodium azide
Purity	Rabbit antiserum
Immunogen	C-terminal peptide of mouse Calmegin, DESPGSGDAPLKSLRKRRVRKD, conjugated with KLH
Isotype	Rabbit IgG
Reactivity	Mouse
Special notes	Validation: Specificity validated with knock-out mouse (Fig.2.)
Application	<ol style="list-style-type: none"> 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)
Background	<p>Calmegin plays an important role in sperm fertility. Binds calcium ions. Specifically expressed during male meiotic germ cell development.</p> <p>Molecular mass: 69,431 with 611 amino acids.</p>
Data Link	uniprot/P52194 mouse Calmegin , Gene ID 12745 mouse Clgn
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

Data Images: 73-034 Anti-Calmegin antibody, rabbit serum

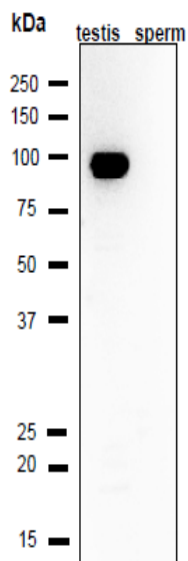


Fig.1 Western blot analysis of Calmegin in lysates of mouse testis and sperm.

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.

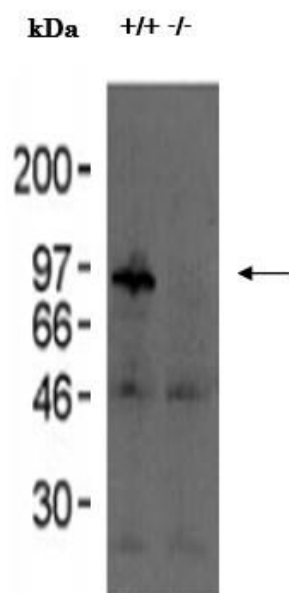


Fig.2 Western blotting analysis of testis extracts of wild-type and knockout mice with anti-Calmegin antibody.

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.

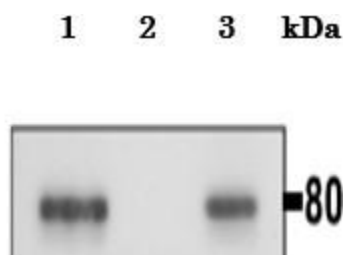


Fig.3. Immunoprecipitation of Calmegin from mouse testis.

One mg of testis lysate was incubated with 2 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

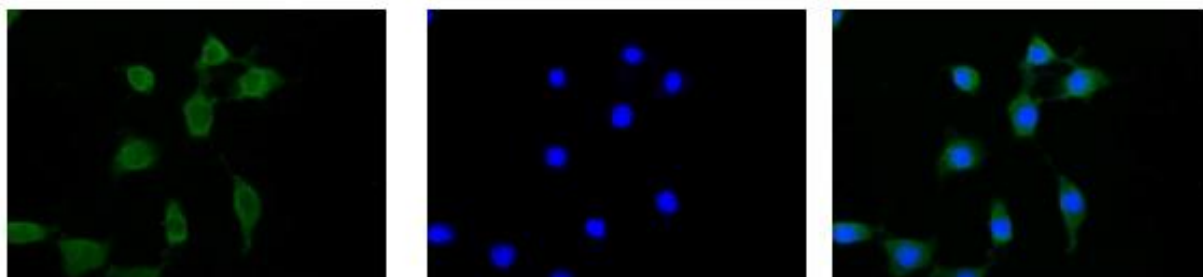


Fig.4 Immunofluorescent staining of Calmegin in NIH3T3 cells with anti-Calmegin antibody

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

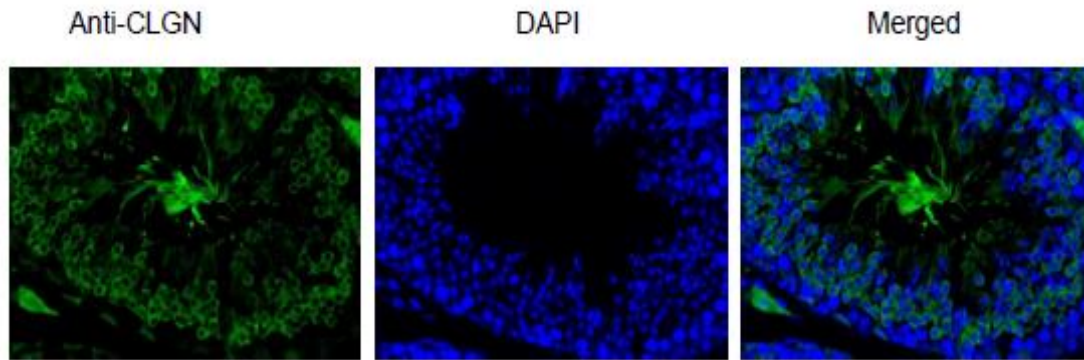


Fig.5 Immunohistological staining of Calmegin in testis section with anti-Calmegin antibody

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