

Anti-Calnexin antibody, rabbit serum

Product code	73-026
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 Calnexin protein, EDEILNRSPNRKPRRE, conjugated with KLH
Isotype	Rabbit IgG
Reactivity	Mouse, rat, human
Special notes	N/A
Application	<ol style="list-style-type: none"> 1. Western blotting (1/1,000 dilution) 2. Immunoprecipitation (1/100 dilution). 3. Immunohistochemistry (1/100 – 1/1,000 dilution) 4. Immuno-fluorescent staining (1/100~1/300 dilution)
Background	<p>Calcium-binding protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins. Associated with partial T-cell antigen receptor complexes that escape the ER of immature thymocytes, it may function as a signaling complex regulating thymocyte maturation. Additionally it may play a role in receptor-mediated endocytosis at the synapse.</p> <p>Molecular mass: 67,278 Da with 591 amino acids. It undergoes palmitoylation and phosphorylation.</p>
Data Link	uniprot/P35564 mouse calnexin. _Gene ID 12330 mouse calnexin.
Please note: All products are FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR MILITARY USE.	

Data Images: 73-026 Anti-Calnexin antibody, rabbit serum

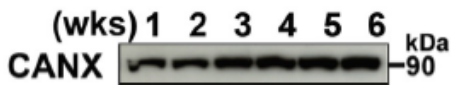


Fig.1 Western blotting analysis of mouse testis extracts of different ages with anti-Calnexin (CANX) antibody.

20 µg of Triton X-100 extracts from mouse testis was reacted with anti-Calnexin antiserum at 1/1,000 dilution.

Calnexin expression started at birth. (wks) stands for weeks.



Fig.2 Western blotting analysis of lysates of mouse testis and sperm with anti-Calnexin (CANX) antibody.

Proteins of the lysates (10 µg) of mouse testis and sperm were separated on SDS-PAGE and blotted to PVDF membrane and reacted with anti-Calnexin antiserum at 1/1,000 dilution. Calnexin bands migrated to the position corresponding to molecular mass of 90 kDa.

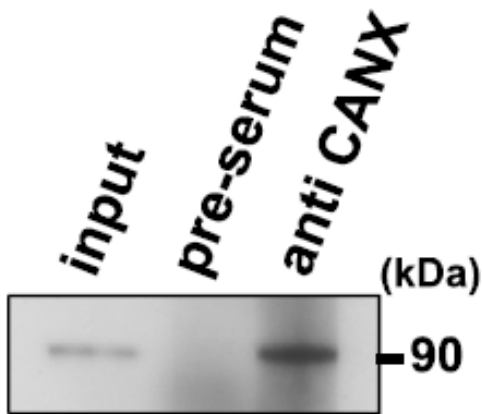


Fig.3.Immunoprecipitation of Calnexin from mouse testis. One mg of testis lysate (supernatant in lysis buffer containing 10 mM Tris-HCl pH8.0, 50 mM NaCl, 1% protease inhibitor mix) was incubated with 2 μ l of anti-Calnexin (CANX) antiserum and 50 μ l. of protein-A conjugated magnetic beads (Miltenyi Biotec) and immunoprecipitated according to the protocol of supplier. The immunoprecipitated sample was analyzed by western blotting using the same antibody at 1/1,000 dilution.

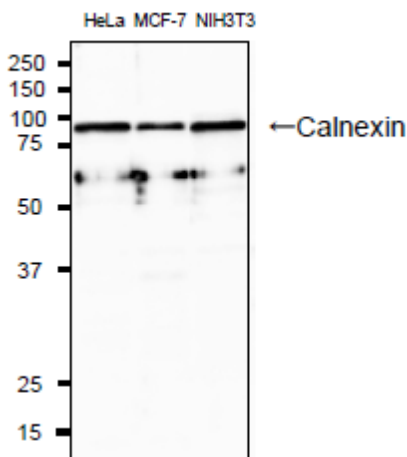


Fig.4 Western blotting analysis of lysates of human and mouse cell lines with anti-Calnexin (CANX) antibody.

Proteins of the lysates (20 μ g) of HeLa, MCF7 and NIH3T3 cells were separated on SDS-PAGE and blotted to PVDF membrane and reacted with anti-Calnexin antiserum at 1/1,000 dilution. As second antibody, goat polyclonal antibody to rabbit IgG conjugated with HRP (ab97051) was used.

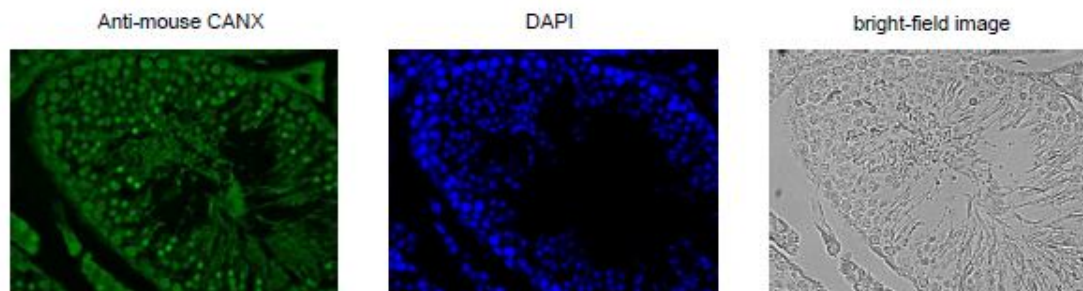
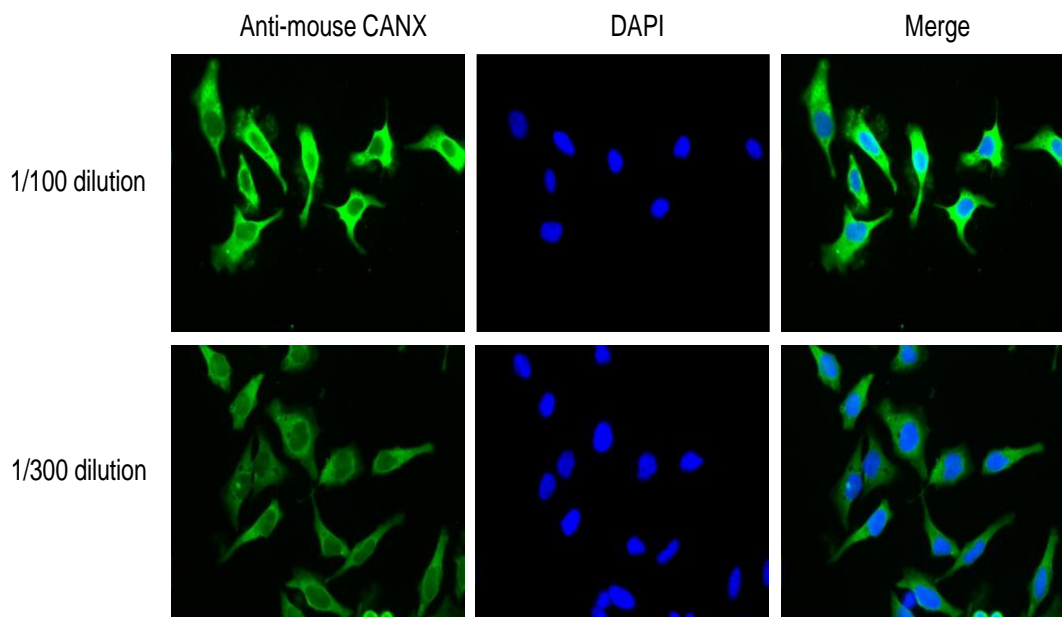


Fig.5. Immunohistochemistry of mouse testis using anti-CANX antibody.

Formalin-fixed and paraffin-embedded mouse testis Deparaffinization by LemosolRA (#122-03991,Wako, Osaka)

Rehydration	100% Et-OH, 95%, 90%, 70%, DW
Antigen retrieval	Histo/Zyme (Cat.# k046; Diagnostic BioSystems)
Washing	PBST (0.25% triton X-100/PBS-)
Blocking	10 % FBS / PBST, 30 min
1st antibody	100 dilution in PBS- 4°C O/N
Washing	PBS-, 5 min, 3times
2nd antibody	1000 dilution, 60 min (Alexa Flour-488 goat anti-rabbit IgG (H&L), #1166843 ; molecular probes)
washing	PBS-, 5 min, 3times
DAPI	1.0µg/mL DAPI in TBS, 10 min
Washing	PBS-, 5 min, 3times
Mount	ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)

Fig.6 Immunofluorescent staining of HeLa cells with anti-Calnexin antibody



wash	PBS-, 2times
fixed	4 % PFA r.t. for 30 min/ 4°C over night
wash	PBS- 5 min X 3 times
permeabilized	0.25 % Triton X-100/PBS 10 min
wash	PBS- , 5 min X 3 times
blocking	1.5% BSA/PBS, 30 min
1st antibody	dilution buffer; 1.5 % BSA/PBS or CanGet signal B 100, 300 or 1000 dilution, 4°C over night (r.t. for 60 min)
wash	PBS- , 5 min X 3 times
2nd antibody	1000 dilution, 60 min (Alex Fluor 488 goat-anti-rabbit IgG (H&L); molecular probes)
wash	PBS- , 5 min X 3 times
DAPI	1.0µg/mL DAPI in TBS, 5 min (X100 stock, 0.1mg/mL)
wash	0.1 % Triton X-100/TBS, 5min TBS, 5min
mount	ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)

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. **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.