

## Anti-Calnexin antibody, rabbit polyclonal

73-026 100  $\mu$ l

**Key words:** Canx, Endoplasmic reticulum, Chaperon, Synaptic vesicle endocytosis, Transmembrane, Calcium ion binding

**Function:** 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

**Molecular mass:** 67,278 Da with 591 amino acids. It undergoes palmitoylation and phosphorylation.

### Applications:

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)

**Immunogen:** C-terminal peptide of mouse Calnexin protein, EDEILNRSRNRKPRRE, conjugated with KLH

**Reactivity:** Mouse, rat, human

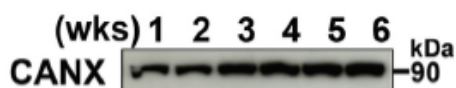
**Form:** Whole rabbit antiserum added with 0.1% sodium azide.

**Storage:** Shipped at 4°C or at -20°C. Upon arrival, spin-down and store at -20°C for longer period.

**Database Links:** [uniprot/P35564](#) mouse calnexin. [Gene ID 12330](#) mouse calnexin.

**Reference:** This antibody was described in Ref.1 and used in the following publications. Ikawa M. et al. (2001) Calmegin Is Required for Fertilin  $\alpha/\beta$  Heterodimerization and Sperm Fertility. [Dev Biol.](#) 240: 254-61. **IP.** Open access.

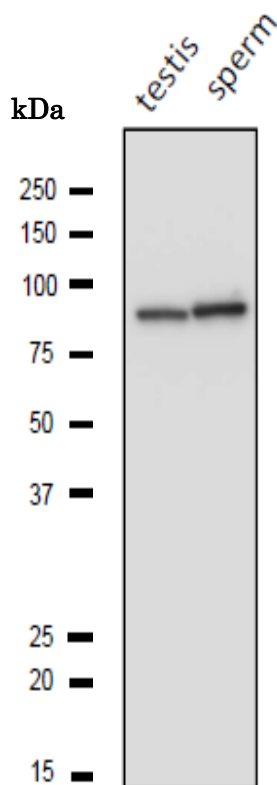
1. Ikawa M. et al. (2011) Calsperin is a testis-specific chaperone required for sperm fertility. [J Biol Chem.](#) 286: 5639-46. **WB, IP.** Open access.



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

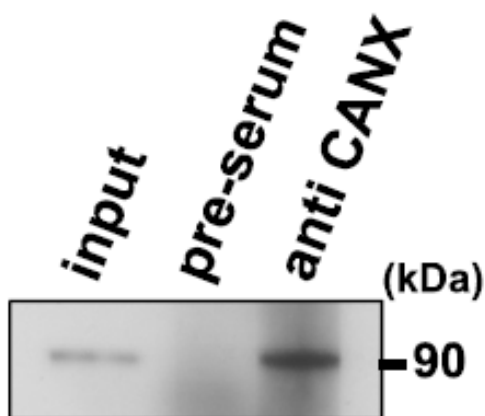
20  $\mu$ g of Triton X-100 extracts from mouse testis was reacted with anti-Calnexin antiserum at 1/1,000 dilution.

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

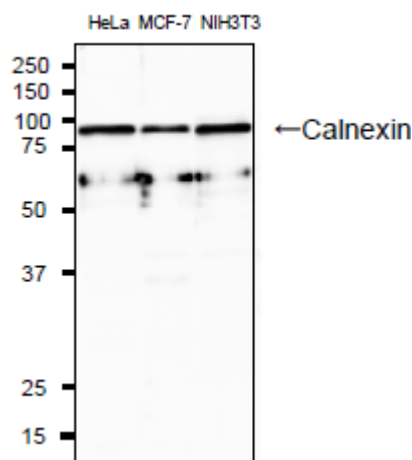


**Fig.2 Western blotting analysis of lysates of mouse testis and sperm with anti-Calnexin (CANX) antibody.**

Proteins of the lysates (10  $\mu$ g) 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.

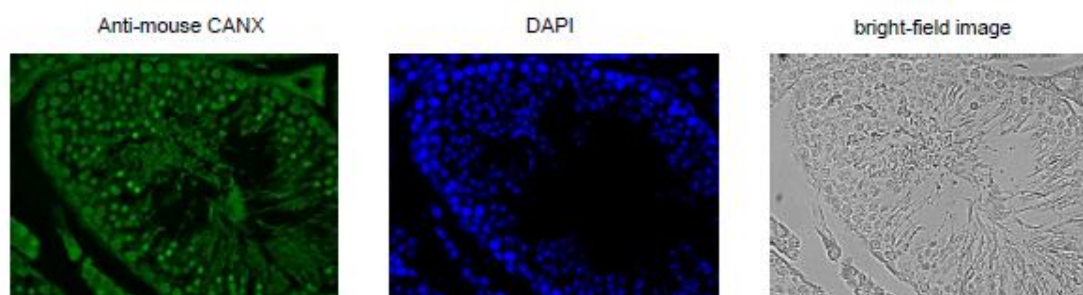


**Fig.3. Immunoprecipitation of Calnexin from mouse testis.** One mg of testis lysate (supernatant in lysis buffer containing 10 m Tris-HCl pH8.0, 50 mM NaCl, 1% protease inhibitor mix) was incubated with 2  $\mu$ l of anti-Calnexin (CANX) antiserum and 50  $\mu$ l of protein-A conjugated magnetic beads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

2nd antibody 1000 dilution, 60 min (Alexa Flour-488 goat anti-rabbit IgG (H&L), #1166843 ; molecular probes)

washing PBS- 5 min X 3

DAPI 1.0  $\mu$ g/mL DAPI in TBS 10 min  
( $\times$  100 stock, 0.1mg/mL)

Washing PBS-

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

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

