

Anti-Calreticulin-3 / CALR3 / Calsperin antibody, rabbit polyclonal

70-022 100 μ l

Key words: Calreticulin-3, CALR3, Calsperin, EIF2 complex, Spermatogenesis, Differentiation, Endoplasmic reticulum

Function: CALR3 capacity for calcium-binding may be absent or much lower than that of CALR. During spermatogenesis, may act as a lectin-independent chaperone for specific client proteins such as ADAM3. Required for sperm fertility.

Molecular mass: 44,232 with 380 amino acids.

Applications:

1. Western blotting (1/1,000 dilution)
2. Immunoprecipitation (1/100 dilution)
3. Immunohistochemistry (1/100 dilution)

Other applications have not been tested.

Immunogen: Synthetic peptide corresponding to C-terminal region of mouse CALR3, CMGKFHRHNHLSRFHRQGEL.

Reactivity: Mouse. Not tested with other species.

Form: Rabbit antiserum added with 0.1% sodium azide

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

Data Links: [uniprot/Q9D9Q6](https://uniprot.org/Q9D9Q6) mouse

[Gene ID 73316](https://ncbi.nlm.nih.gov/gene/73316) mouse

Reference: This antibody was described and used in the following publication.

Ikawa M. et al (2011) Calsperin is a testis-specific chaperone required for sperm fertility. J Biol Chem. 18:5639-46. [pubmed/21131354](https://pubmed.ncbi.nlm.nih.gov/21131354/) **WB, IP, IHC.** Free article.

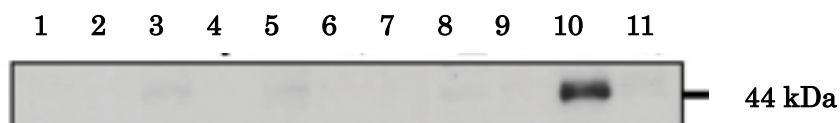


Fig. 1. Testeis specific expression of Calreticulon-3 as examined in various tissues by western blotting with anti-CALR3 antibody. The various tissues were excised and homogenized in lysis buffer containing 1% TritonX100 and then placed on ice for 1 h. These extracts were centrifuged, and the supernatants were collected and analyzed by western blotting with anti-CALR3 antibody at 1/1,000 dilution.

1. Brain.
2. Lung.
3. Heart.
4. Thymus.
5. Liver.
6. Spleen.
7. Kidney.
8. Muscle.
9. Ovary.
10. Testis.
11. Sperm

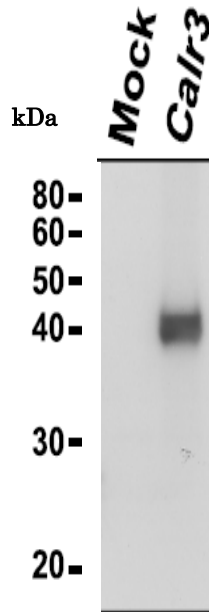


Fig.2. Identification of Calreticulon-3 protein by western blotting with anti-CALR3 antibody.

Embryonic fibroblast cells prepared from *Calr3*^{-/-} mouse were transfected with a plasmid expressing *Calr3*. The cell lysate was analyzed by western blotting with anti-CALR3 antibody at 1/1,000 dilution.

1. Mock-infected cell lysate as a negative control.
2. Cell lysate transfected with a plasmid expressing *Calr3*.

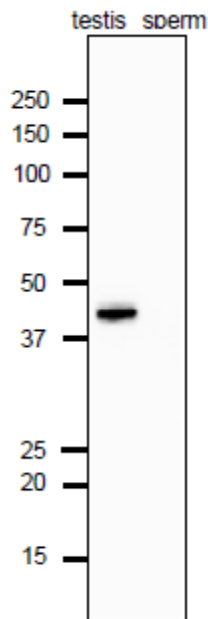


Fig.3. Analysis of Calreticulon-3 protein in the extracts of mouse testis and sperm by western blotting with anti-Calreticulon-3 antibody.

Proteins in the extracts (10 μ g protein) were separated on SDS-PAGE (10-20% gradient gel), electro-blotted to PVDF membrane and reacted with anti-Calreticulon-3 antibody at 1/1,000 dilution. As the second antibody, anti-rat IgG antibody conjugated with HRP (ab97051) was used at 1/10,000 dilution. The numbers on the left are positions of protein size markers in kDa.

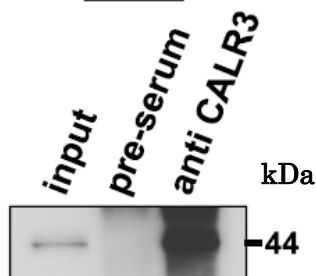


Fig.4. Immunoprecipitation of Calreticulon-3 protein with anti-CALR3 antibody.

Lysates of wild-type mouse testis were immunoprecipitated with anti-CALR3 antibody and the precipitates were analyzed by western blotting with the same antibody.

1. Input testis lysate
2. Precipitated with preimmune serum
3. Precipitated with anti-CALR3 antibody

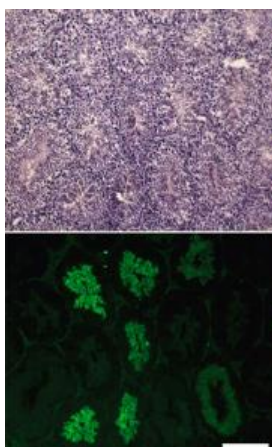


Fig.5. Immunofluorescence staining of a testicular section with anti-CALR3 antibody. Sequential sections were stained with hematoxylin and eosin (upper panel). CALR3 was detected in elongating spermatids (lower panel). Testis was collected from adult mouse and fixed in 4% paraformaldehyde/PBS overnight at 4 °C, cryopreserved in graded 10–30% sucrose, and embedded. Frozen sections (8 μ m) were mounted on aminopropyltriethoxysilane-coated glass slides. Primary antibody was used at 1/100 and as secondary antibody, Alexa Fluor 488 conjugated goat anti-rabbit IgG was used. Scale bar is 200 μ m.

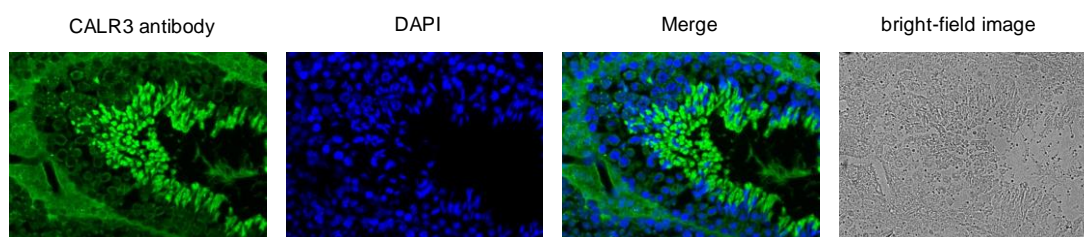


Fig.6 Immunohistochemistry of formalin fixed and paraffin embedded mouse testis

Deparaffinization	Lemosol ^{RA} (#122-03991,Wako, Osaka)
rehydration	
antigen retrieval	Histo/Zyme (Cat.# k046; Diagnostic BioSystems)
Washing	PBST (0.25% triton X-100/PBS-)
Blocking	1 % BSA / PBST 60 min
1 st antibody	100, 1000 dilution in PBS- 4°C O/N
Washing	PBS-
2 nd antibody	1000 dilution, 60 min
washing	PBS- 5 min X 3
DAPI	1.0 μ g/mL DAPI in TBS 10 min (\times 100 stock, 0.1mg/mL)
Washing	PBS-
Mount	ImmunoSelect Antifading Mounting Medium (SCR-38447; Dianova)