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

73-022　　100 µl

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

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

Form: Rabbit antiserum added with 0.1% sodium azide

Reactivity: Mouse.　Not tested with other species.

Applications:

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

Other applications have not been tested.

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

Background: 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.

Data Links: uniprot/Q9D9Q6 mouse

Gene ID 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 **WB, IP, IHC.** Free article.

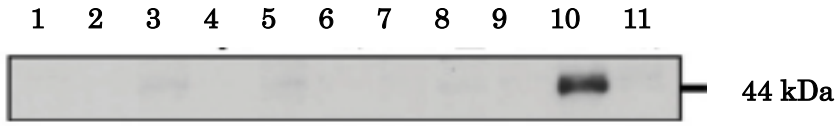

Fig. 1. Testeis specific expression of Calreticulon-3 as examined in various tissues by western blotting with anti-CALR3 antibody. The various tissues were excised and homogenized in lysis buffer containing 1% TritonX100 and then placed on ice for 1 h. These extracts were centrifuged, and the supernatants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

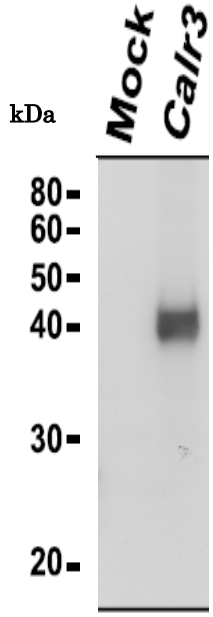

Fig.2. Identification of Calreticulon-3 protein by western blotting with anti-CALR3 antibody.

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*.

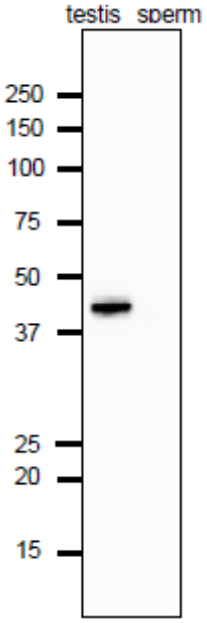

Fig.3. Analysis of Calrecticulon-3 protein in the extracts of mouse testis and sperm by western blotting with anti-Calrecticulon-3 antibody.

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

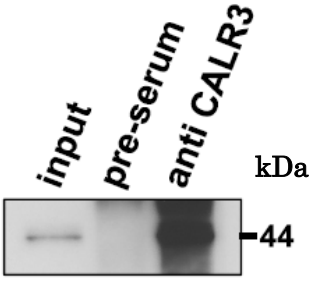

Fig.4. Immunoprecipitation of Calreticulon-3 protein with anti-CALR3 antibody.

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

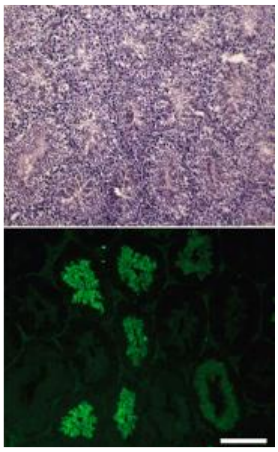

Fig.5. Immunofluorescence staining of a testicular section with anti-CALR3 antibody. Sequential sections were stained with hematoxylin and eosin (upper panel). CALR3 was detected in elongating spermatids (lower panel). Testis was collected from adult mouse and fixed in 4% paraformaldehyde/PBS overnight at 4 °C, cryopreserved in graded 10–30% sucrose, and embedded. Frozen sections (8 μm) 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 μm.