

Anti-Calreticulin / CALR antibody, rabbit serum

73-018 100 μl

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

Immunogen: C-EEDEKEEDEEESPGQAKDEL (C-terminal of mouse CALR protein)

Validation: Knock-out mouse

Form: Whole rabbit antiserum added with 0.09% sodium azide.

Reactivity: Mouse and human. Not tested with other species.

Applications:

1. Western blotting (1/500~1/1,000 dilution))

- 2. Immunoprecipitation (1/100)
- 3. Immunoflourescence staining (1/300~1/1,000 dilution)
- 4. Immunohistochemistry (1/1,000 dilution)

Other applications have not been tested.

Key words: Calreticulin, CALR, XCRP55, Calregulin, Endoplasmic reticulum resident protein 60 (ERp60), Lectin chaperon, calcium homeostasis

Background: Calreticulin (CALR) is calcium-binding chaperone that promotes folding, oligomeric assembly and quality control in the endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. This lectin interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER. Interacts with the DNA-binding domain of NR3C1 and mediates its nuclear export. Involved in maternal gene expression regulation. May participate in oocyte maturation via the regulation of calcium homeostasis

Data Links: uniprot/P14211 Mouse Calrecticulin, uniprot/Q96L12 Human Calreticulin

Gene I D 12317 Mouse Calr, Gene ID 811 Human Calr

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 Free article. **WB, IP**,

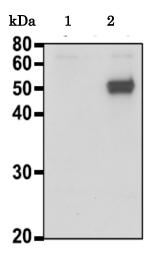


Fig.1 Identification of CALR protein by western blotting with anti-CALR antibody.

Embryonic fibroblast cells prepared from *Calr -/-* mouse were transfected with a plasmid expressing *Calr*. The cell lysate was analyzed by western blotting with anti-CALR antibody at 1/500 dilution.

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

The molecular mass is 48 kDa



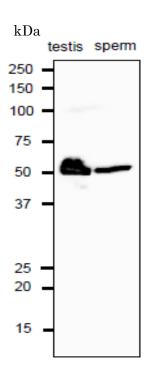


Fig.2 Western blot analysis of CALR protein in crude extracts of mouse testis and sperm with anti-CALR antibody.

Proteins in the extracts (10 μ g protein) were separated on 10-20% gradient gel of SDS-PAGE and electro-blotted to a PVDF membrane. The membrane was reacted with anti-CALR antibody at 1/1,000 dilution. As the 2nd antibody, anti-rat IgG antibody conjugated with HRP (ab97051) was used at 1/10,000 dilution

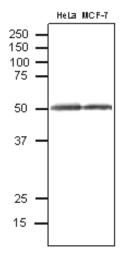


Fig.3 Western blot analysis of CALR protein in crude extracts of human cell lines with anti-CALR antibody.

Proteins in the extracts (10 μ g protein) were separated on 12.5% gel of SDS-PAGE and electro-blotted to a PVDF membrane (wet system). The membrane was reacted with anti-CALR antibody at 1/1,000 dilution. As the 2nd antibody, anti-rat IgG antibody conjugated with HRP (ab97051) was used at 1/10,000 dilution



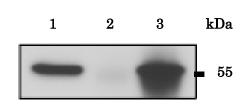


Fig.4. Immunoprecipitation of CALR protein with anti-CALR antibody.

Lysates of wild-type mouse testis were immunoprecipitated with anti-CALR 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-CALR antibody

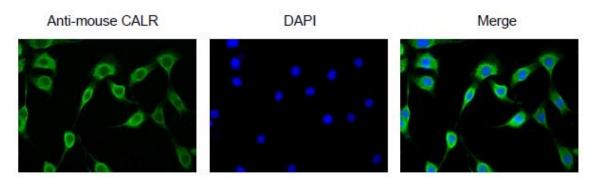


Fig.5 Immunofluorescence staining of CALR protein in NIH3T3 cells with anti-CALR antibody.

NIH3T3 cells were fixed with 4% paraformal dehyde and permeabilized with 0.5% TritonX 100 and reacted with anti-CALR antibody at 1/300 dilution. As the $2^{\rm nd}$ antibody, goat anti-rabbit IgG antibody conjugated with Alexa Fluor 488 (Molecular Probes) was used at 1/1,000 dilution.

DNA was stained with DAPI (1 ug/ml) and the merged image was shown on right.

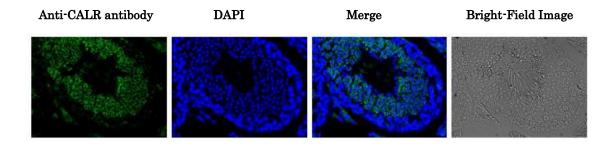


Fig.6. Immunohistological staining of CALR protein in mouse testis using anti-CALR antibody.

A section of formalin fixed and paraffin embedded mouse testis was treated with the anti-CALR antibody at 1/1,000 dilution after deparaffization and antigen retrieval. The $2^{\rm nd}$ antibody, goat anti-rabbit IgG conjugated with Alexa Fluor 488 (Molecular Probes #1166843) was used at 1/1,000 dilution. DNA was stained with DAPI (1.0 μ g/mL) and the merged image was shown (Merge). The bright-field image of the same region was shown on the right.