Anti-Calnexin Antibody

ER1803-42

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 68 kDa

Description: Calnexin and Calregulin (also called calreticulin) are calcium-binding proteins that are localized to the endoplasmic reticulum, Calnexin to the membrane and Calregulin to the lumen. Calnexin is a type I membrane protein that interacts with newly synthesized glycoproteins in the endoplasmic reticulum. It may play a role in assisting with protein assembly and in retaining unassembled protein subunits in the endoplasmic reticulum. Calregulin has both low- and high-affinity calcium-binding sites. Neither Calnexin nor Calregulin contains the calcium-binding “E-F hand” motif found in calmodulins. Calnexin and Calregulin are important for the maturation of glycoproteins in the endoplasmic reticulum and appear to bind many of the same proteins.

Immunogen: Synthetic peptide within human Calnexin aa 555-592/592.

Positive control: Daudi cell lysate, mouse lung tissue lysate, human kidney tissue, SKOV-3, Hela cell lysate, rat lung tissue lysate, rat brain tissue lysate, rat brain tissue, human brain tissue, mouse brain tissue.

Subcellular location: Endoplasmic reticulum membrane, Endoplasmic reticulum, Melanosome.

Database links: SwissProt: P27824 Human | P35564 Mouse

Recommended Dilutions:

WB	1:2,000- 1:10,000
IHC-P	1:200-1:500
FC	1:50-1:100
IP	1-2μg/sample

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

Images

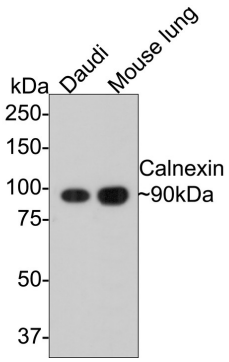

Fig1: Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (ER1803-42) at 1/5,000 dilution.

Lane 1: Daudi cell lysate (10 µg/Lane)
Lane 2: Mouse lung tissue lysate (20 µg/Lane)

Lysates/proteins at 10 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 90 kDa

Exposure time: 2 minutes;

8% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1803-42) at 1/5,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

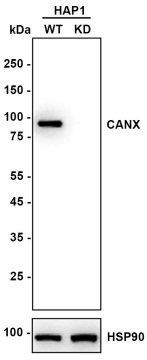

Fig2: Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (ER1803-42) at 1/10,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-Calnexin KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 90 kDa

Exposure time: 20 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1803-42) at 1/10,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

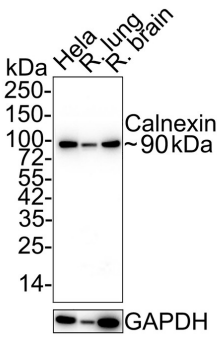

Fig3: Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (ER1803-42) at 1/2,000 dilution.

Lane 1: Hela cell lysate（10 µg/Lane）
Lane 2: Rat lung tissue lysate（20 µg/Lane）
Lane 3: Rat brain tissue lysate（20 µg/Lane）

Predicted band size: 68 kDa
Observed band size: 90 kDa

Exposure time: 3 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ER1803-42) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

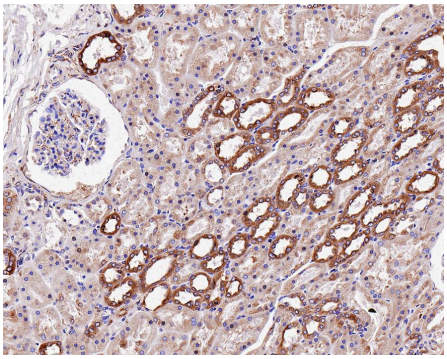

Fig4: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Calnexin antibody (ER1803-42) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1803-42) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

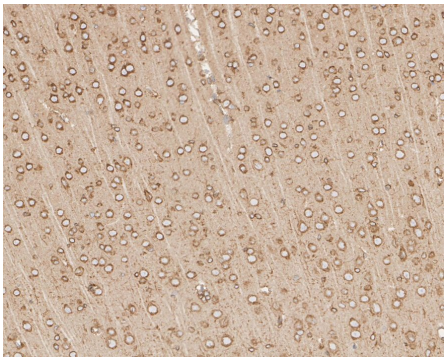

Fig5: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Calnexin antibody (ER1803-42) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1803-42) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

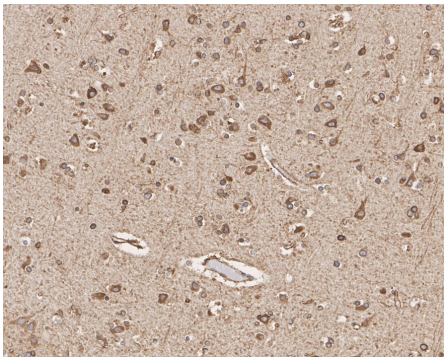

Fig6: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-Calnexin antibody (ER1803-42) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1803-42) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

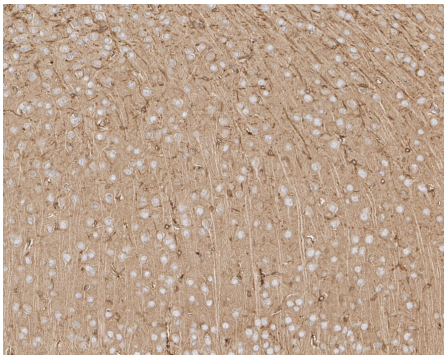

Fig7: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-Calnexin antibody (ER1803-42) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ER1803-42) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

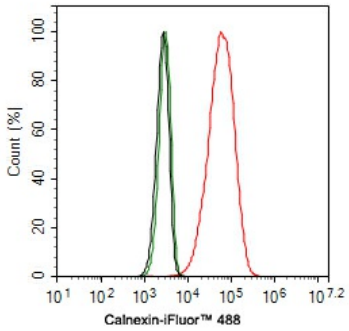

Fig8: Flow cytometric analysis of SKOV-3 cells labeling Calnexin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER1803-42, 1ug/ml) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

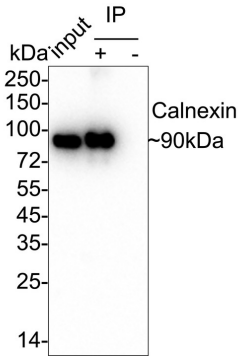

Fig9: Calnexin was immunoprecipitated from 0.2 mg HeLa cell lysate with ER1803-42 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ER1803-42 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ER1803-42 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ER1803-42 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 23 seconds; ECL: K1801

Background References

1. Lakkaraju A K et al. Palmitoylated calnexin is a key component of the ribosome-translocon complex. EMBO J 31:1823-1835 (2012).
2. Basrur V et al. Proteomic analysis of early melanosomes: identification of novel melanosomal proteins. J Proteome Res 2:69-79 (2003).