Anti-Calreticulin Antibody [SU37-03]

ET1608-60

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	SU37-03

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 Calreticulin aa 40-89 / 417.

Positive control: HepG2 cell lysate, HeLa cell lysate, HL-60 cell lysate, C2C12 cell lysate, C6 cell lysate, Mouse liver tissue lysate, Rat liver tissue lysate, HeLa, human liver tissue, human liver carcinoma tissue, human kidney tissue, mouse brain tissue.

Subcellular location: Endoplasmic reticulum lumen, Cytoplasm, Secreted, Cell surface, Sarcoplasmic reticulum lumen, nucleus.

Database links: SwissProt: P27797 Human | P14211 Mouse | P18418 Rat

Recommended Dilutions:

WB	1:50,000
IF-Cell	1:2,000
IHC-P	1:5,000
FC	1:2,000
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

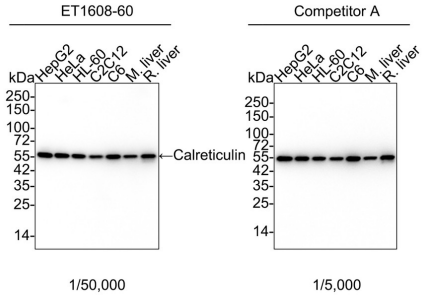


Fig1: Western blot analysis of Calreticulin on different lysates with Rabbit anti-Calreticulin antibody (ET1608-60) at 1/50,000 dilution and competitor's antibody at 1/5,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: HeLa cell lysate
Lane 3: HL-60 cell lysate
Lane 4: C2C12 cell lysate
Lane 5: C6 cell lysate
Lane 6: Mouse liver tissue lysate
Lane 7: Rat liver tissue lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 1 minute 2 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 (ET1608-60) at 1/50,000 dilution and competitor's antibody at 1/5,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

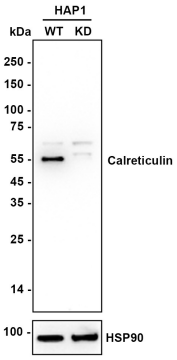


Fig2: Western blot analysis of Calreticulin on different lysates with Rabbit anti-Calreticulin antibody (ET1608-60) at 1/50,000 dilution.

Lane 1: HAP1-parental cell lysate (10 µg/Lane)
Lane 2: HAP1-Calreticulin KD cell lysate (10 µg/Lane)

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 30 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 (ET1608-60) at 1/50,000 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.

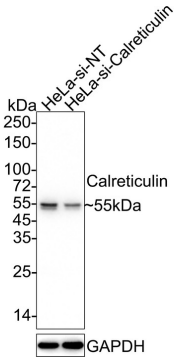


Fig3: Western blot analysis of Calreticulin on different lysates with Rabbit anti-Calreticulin antibody (ET1608-60) at 1/50,000 dilution.

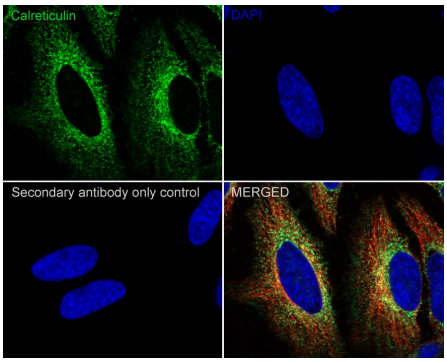
Lane 1: HeLa-si NT cell lysate (10 µg/Lane)
Lane 2: HeLa-si Calreticulin cell lysate (10 µg/Lane)

Predicted band size: 48 kDa
Observed band size: 55 kDa

Exposure time: 13 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 (ET1608-60) at 1/50,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig4: Immunocytochemistry analysis of HeLa cells labeling Calreticulin with Rabbit anti-Calreticulin antibody (ET1608-60) at 1/2,000 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Calreticulin antibody (ET1608-60) at 1/2,000 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

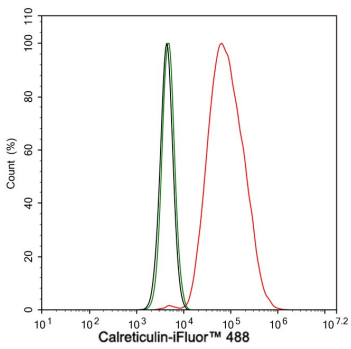


Fig5: Flow cytometric analysis of HeLa cells labeling Calreticulin.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1608-60, 1/2,000) (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).

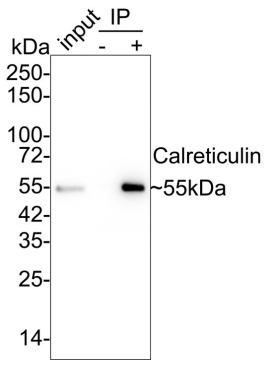


Fig6: Calreticulin was immunoprecipitated in 0.2mg HeLa cell lysate with ET1608-60 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using ET1608-60 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: Rabbit IgG instead of ET1608-60 in HeLa cell lysate
Lane 3: ET1608-60 IP in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 24 seconds

Background References

1. Kojima Y et al. Cyclin-dependent kinase inhibitor 2B regulates efferocytosis and atherosclerosis. J Clin Invest 124:1083-97 (2014).
2. Angelova AL et al. Complementary induction of immunogenic cell death by oncolytic parvovirus H-1PV and gemcitabine in pancreatic cancer. J Virol 88:5263-76 (2014).