Anti-Calnexin Antibody [SN20-54] - BSA and Azide free HA750275



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IP, IHC-P, IF-Tissue

Molecular Wt: Predicted band size: 68 kDa

Clone number: SN20-54

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 543-592 / 592.

Positive control: HeLa cell lysate, HepG2 cell lysate, MCF7 cell lysate, PANC-1 cell lysate, HAP1 cell lysate,

human kidney tissue, human pancreas tissue, mouse kidney tissue, mouse liver tissue, rat

kidney tissue, rat liver tissue, rat brain tissue.

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

Database links: SwissProt: P27824 Human | P35564 Mouse | P35565 Rat

Recommended Dilutions:

 $\begin{array}{lll} \mbox{WB} & 1:2,000\mbox{-}1:10,000 \\ \mbox{IP} & 1\mbox{-}2\mu\mbox{g/sample} \\ \mbox{IHC-P} & 1:1,000\mbox{-}1:10,000 \\ \end{array}$

IF-Tissue 1:1,000

Storage Buffer: PBS (pH7.4).

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

cycles.

Purity: Protein A affinity purified.

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Images

kDa 250-250-150-100-72-55-42-35-25-14**Fig1:** Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (HA750275) at 1/2,000 dilution.

Lane 1: HeLa cell lysate Lane 2: HepG2 cell lysate Lane 3: MCF7 cell lysate Lane 4: PANC-1 cell lysate

Lysates/proteins at 15 µg/Lane.

Predicted band size: 68 kDa Observed band size: 100 kDa

Exposure time: 2 minutes;

4-20% SDS-PAGE gel.

Fig2: Western blot analysis of Calnexin on different lysates with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 dilution.

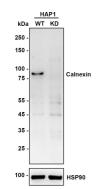
Lane 1: HAP1-parental cell lysate Lane 2: HAP1-Calnexin KD cell lysate

Lysates/proteins at 10 µg/Lane.

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

Exposure time: 180 seconds; ECL: K1802;

4-20% SDS-PAGE gel.



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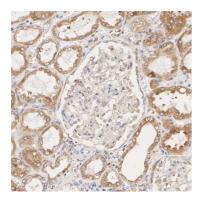


Fig3: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/10,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/10,000 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.

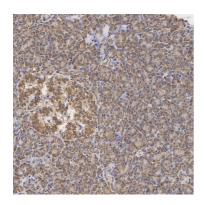


Fig4: Immunohistochemical analysis of paraffin-embedded human pancreas tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/2,000 dilution.

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 $_2$ O and PBS, and then probed with the primary antibody (HA750275) at 1/2,000 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.

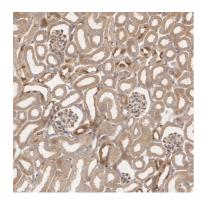


Fig5: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/1,000 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.

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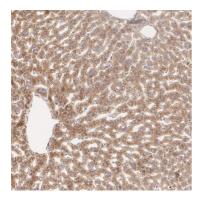


Fig6: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/1,000 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.

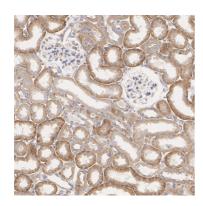


Fig7: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/1,000 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: Immunohistochemical analysis of paraffin-embedded rat liver tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/1,000 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.

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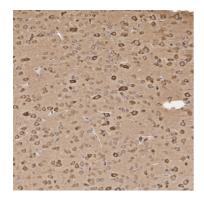


Fig9: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-Calnexin antibody (HA750275) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA750275) at 1/1,000 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.

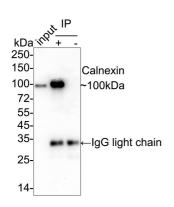


Fig10: Calnexin was immunoprecipitated from 0.2 mg HeLa cell lysate with HA750275 at 2 μ g/10 μ l beads. Western blot was performed from the immunoprecipitate using HA750275 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)

Lane 2: HA750275 IP in HeLa cell lysate

Lane 3: Rabbit IgG instead of HA750275 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 1 minute 5 seconds; ECL: K1801

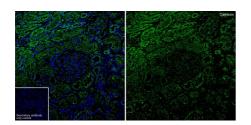


Fig11: Application: IF-Tissue

Species: Human

Site: kidney

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

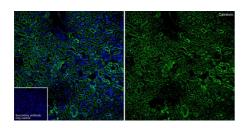


Fig12: Application: IF-Tissue

Species: Mouse

Site: kidney

Sample: Paraffin-embedded section

Antibody concentration: 1/1,000

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- Noy PJ et al. TspanC8 Tetraspanins and A Disintegrin and Metalloprotease 10 (ADAM10) Interact via Their Extracellular Regions: EVIDENCE FOR DISTINCT BINDING MECHANISMS FOR DIFFERENT TspanC8 PROTEINS. J Biol Chem 291:3145-57 (2016).
- 2. Askautrud HA et al. Global gene expression analysis reveals a link between NDRG1 and vesicle transport. PLoS One 9:e87268 (2014).