sigma-aldrich.com

3050 Spruce Street, St. Louis, MO 63103 USA Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757 email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-Calnexin

produced in rabbit, IgG fraction of antiserum

Catalog Number C4731

Product Description

Anti-Calnexin is produced in rabbit using as immunogen a synthetic peptide corresponding to the C-terminus of human calnexin (amino acids 573-592) conjugated to KLH. This sequence is identical in dog, rat, and mouse. Whole antiserum is purified to provide an IgG fraction of antiserum.

Anti-Calnexin recognizes human, dog, rat and mouse calnexin (90 kDa) in immunoblotting, immunoprecipitation, and immunofluorescence applications. Staining of calnexin in immunoblotting is specifically inhibited with the calnexin immunizing peptide.

Calnexin (p88, IP90), is a calcium-binding, type I integral membrane protein, localized primarily in the endoplasmic reticulum (ER).¹⁻⁵ Newly synthesized cellular and extracellular proteins must be correctly folded and assembled in the ER before they progress to the cytosol or cell surface. This process is facilitated by transient interaction with a specific set of chaperones that reside in the ER lumen including calnexin, calreticulin, protein disulfide isomerase (PDI), and molecular chaperones of the Hsp60, Hsp70, and Hsp90 families. Calnexin binds newly synthesized glycoproteins and misfolded proteins, and is believed to play a critical role in quality control processes during protein synthesis and folding. Calnexin acts as a lectin-like chaperone that binds oligosaccharide residues of newly synthesized N-linked glycoproteins. The lectin specificity of calnexin, and its soluble homologue calreticulin, has been identified as high mannose oligosaccharides terminating in monoglucosyl residues linked through α 1-3.^{1,5-8} Calnexin has been shown to be associated with several cell surface proteins, including MHC class I heavy chain, T-cell receptor (TCR), and B cell membrane immunoglobulin during translocation through the ER.⁹⁻¹² It also forms complexes with other resident ER proteins involved in Ca²⁺-dependent retention of proteins.

Calnexin contains a long (461 amino acids) N-terminal Ca²⁺-binding domain extending into the lumen of the ER, a short (22 amino acids) transmembrane segment, and an acidic cytosolic domain (96 amino acids). These features distinguish calnexin from soluble ER chaperones that cannot interact with the transmembrane and cytosolic domains of integral membrane proteins. The amino acid sequence of calnexin is highly conserved among species, and shares regions of high sequence homology with calreticulin.¹³

Reagent

Supplied in a solution of 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in "frost-free" freezers, is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile

<u>Immunoblotting</u>: a minimum working dilution of 1:2,000 is determined using a RIPA lysate of the human hepatocytoma HepG2 cell line or of the human epitheloid carcinoma HeLa cell line.

The antibody (at least 40 μg of lgG) immunoprecipitates calnexin from a RIPA lysate of the human epitheloid carcinoma HeLa cell line.

<u>Immunofluorescence</u>: a minimum working dilution of 1:200 is determined using the Madin-Darby canine kidney (MDCK) cell line.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

- 1. Bergeron, J.J.M., et al., *Trends Biol. Sci.*, **19**, 124 (1994).
- 2. Wada, I., et al., J. Biol. Chem., 266, 19599 (1991).
- 3. Ou, W.J., et al., J. Biol. Chem., 270, 18051 (1995).
- 4. Hauri, H-P., et al., *FEBS Lett.*, **476**, 32 (2000).

- 5. Ou, W.J., et al., *Nature*, **364**, 771 (1993).
- Hammond, C., et al., *Proc. Natl. Acad. Sci. USA*, 91, 913 (1994).
- 7. Hebert, D.N., et al., *Cell*, **81**, 425 (1995).
- 8. Ware, F.E., et al., J. Biol. Chem., 270, 4697 (1995).
- 9. Galvin, K., et al., *Proc. Natl. Acad. Sci. USA*, **89**, 8452 (1992).
- 10. Jackson, M.R., et al., *Science*, **263**, 384 (1994).
- 11. Rajagopalan, S., et al., Science, 263, 387 (1994).
- 12. Wada, I., et al., J. Biol. Chem., 269, 7464 (1994).
- 13. Tjoelker, L.W., et al., *Biochemistry*, **33**, 3229 (1994).

MG,KAA,PHC 02/09-1

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.