

Calregulin siRNA (r): sc-63293

BACKGROUND

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.

REFERENCES

1. Smith, M.J. and Koch, G.L. 1989. Multiple zones in the sequence of calreticulin (CRP55, Calregulin, HACBP), a major calcium binding ER/SR protein. *EMBO J.* 8: 3581-3586.
2. David, V., et al. 1993. Interaction with newly synthesized and retained proteins in the endoplasmic reticulum suggests a chaperone function for human integral membrane protein IP90 (Calnexin). *J. Biol. Chem.* 268: 9585-9592.
3. Tjoelker, L.W., et al. 1994. Human, mouse, and rat Calnexin cDNA cloning: identification of potential calcium binding motifs and gene localization to human chromosome 5. *Biochemistry* 33: 3229-3236.
4. Breier, A. and Michalak, M. 1994. 2,4,6-Trinitrobenzenesulfonic acid modification of the carboxyl-terminal region (C-domain) of calreticulin. *Mol. Cell. Biochem.* 130: 19-28.
5. Wada, I., et al. 1995. Chaperone function of calreticulin when expressed in the endoplasmic reticulum as the membrane-anchored and soluble forms. *J. Biol. Chem.* 270: 20298-20304.

CHROMOSOMAL LOCATION

Genetic locus: Calr (rat) mapping to 19q11.

PRODUCT

Calregulin siRNA (r) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Calregulin shRNA Plasmid (r): sc-63293-SH and Calregulin shRNA (r) Lentiviral Particles: sc-63293-V as alternate gene silencing products.

For independent verification of Calregulin (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63293A, sc-63293B and sc-63293C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Calregulin siRNA (r) is recommended for the inhibition of Calregulin expression in rat cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Calregulin (F-4): sc-373863 is recommended as a control antibody for monitoring of Calregulin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Calregulin gene expression knockdown using RT-PCR Primer: Calregulin (r)-PR: sc-63293-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.