

CALR3 siRNA (h): sc-97334

BACKGROUND

Members of the calreticulin (CRT) family are calcium-binding chaperones that are localized to the endoplasmic reticulum or sarcoplasmic reticulum in eukaryotes. Plant CRTs seem to have different properties than their animal counterparts and may be significant during growth and development, as well as biotic and abiotic stress responses. Calreticulin-3, also called CRT2 (calreticulin-2), is a 384 amino acid endoplasmic reticular (ER) protein that contains two calcium binding domains, a P-domain and a C-domain. Calreticulin-3 assists in the calreticulin/calnexin cycle, where it is involved in protein-folding, oligomeric assembly and quality control in the ER. With specific expression in testis, CALR3 has been implicated as a cancer-testis antigen due to its frequent expression in various cancers.

REFERENCES

1. Michalak, M., et al. 1998. Calreticulin, a multifunctional Ca^{2+} binding chaperone of the endoplasmic reticulum. *Biochem. Cell Biol.* 76: 779-785.
2. Michalak, M., et al. 1999. Calreticulin: one protein, one gene, many functions. *Biochem. J.* 344: 281-292.
3. Corbett, E.F., et al. 2000. The conformation of calreticulin is influenced by the endoplasmic reticulum luminal environment. *J. Biol. Chem.* 275: 27177-27185.
4. Persson, S., et al. 2002. Identification of a novel calreticulin isoform (CRT2) in human and mouse. *Gene* 297: 151-158.
5. Persson, S., et al. 2003. Phylogenetic analyses and expression studies reveal two distinct groups of calreticulin isoforms in higher plants. *Plant Physiol.* 133: 1385-1396.

CHROMOSOMAL LOCATION

Genetic locus: CALR3 (human) mapping to 19p13.11.

PRODUCT

CALR3 siRNA (h) 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CALR3 shRNA Plasmid (h): sc-97334-SH and CALR3 shRNA (h) Lentiviral Particles: sc-97334-V as alternate gene silencing products.

For independent verification of CALR3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-97334A, sc-97334B and sc-97334C.

RESEARCH USE

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

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

APPLICATIONS

CALR3 siRNA (h) is recommended for the inhibition of CALR3 expression in human 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 μM in 66 μl . 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

CALR3 (1H2): sc-134295 is recommended as a control antibody for monitoring of CALR3 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 CALR3 gene expression knockdown using RT-PCR Primer: CALR3 (h)-PR: sc-97334-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.