SANTA CRUZ BIOTECHNOLOGY, INC.

CBARA1 siRNA (h): sc-90788

BACKGROUND

The EF-hand domain is a 12 amino acid loop motif that is commonly found in proteins that participate in calcium binding events within the cell. EF-hand domains generally exist in a pair that together form a stable four-helix bundle that enables the binding of calcium ions. CBARA1 (calcium binding atopy-related autoantigen 1), also known as CALC or EFHA3, is a 476 amino acid single-pass membrane protein that contains two EF-hand domains. Expressed at high levels in epidermal keratinocytes and dermal endothelial cells, CBARA1 functions to induce T cell-mediated autoreactivity, which is accompanied by the release of IFN-γ and can induce an allergic reaction that leads to the formation of IgE. IgE can bind to otherwise innocuous environmental particles and, upon binding, can induce cross-linking with an IgE receptor, an event that is associated with atopic dermatitis (AD). Multiple isoforms of CBARA1 exist due to alternative splicing events.

REFERENCES

1. Moncrief, N.D., Kretsinger, R.H. and Goodman, M. 1990. Evolution of EF-hand calcium-modulated proteins. I. Relationships based on amino acid sequences. J. Mol. Evol. 30: 522-562.
2. Nakayama, S., Moncrief, N.D. and Kretsinger, R.H. 1992. Evolution of EF-hand calcium-modulated proteins. II. Domains of several subfamilies have diverse evolutionary histories. J. Mol. Evol. 34: 416-448.
3. Maruyama, K. and Sugano, S. 1994. Oligo-capping: a simple method to replace the cap structure of eukaryotic mRNAs with oligoribonucleotides. Gene 138: 171-174.
4. Kawasaki, H. and Kretsinger, R.H. 1995. Calcium-binding proteins 1: EF-hands. Protein Profile 2: 297-490.
5. Natter, S., Seiberler, S., Hufnagl, P., Binder, B.R., Hirschl, A.M., Ring, J., Abeck, D., Schmidt, T., Valent, P. and Valenta, R. 1998. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. FASEB J. 12: 1559-1569.

CHROMOSOMAL LOCATION

Genetic locus: MICU1 (human) mapping to 10q22.1.

PRODUCT

CBARA1 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 CBARA1 shRNA Plasmid (h): sc-90788-SH and CBARA1 shRNA (h) Lentiviral Particles: sc-90788-V as alternate gene silencing products.

For independent verification of CBARA1 (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-90788A, sc-90788B and sc-90788C.

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

CBARA1 siRNA (h) is recommended for the inhibition of CBARA1 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

CBARA1 (D-10): sc-518183 is recommended as a control antibody for monitoring of CBARA1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CBARA1 gene expression knockdown using RT-PCR Primer: CBARA1 (h)-PR: sc-90788-PR (20 µl). 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.