

CARF siRNA (h): sc-72795

BACKGROUND

CARF (calcium-response factor), also known as ALS2CR8 (amyotrophic lateral sclerosis 2 chromosomal region candidate gene 8 protein) or NYD-SP24, is a 725 amino acid nuclear protein. Expressed in a wide variety of tissues with highest expression in the hippocampus, CARF is thought to be a transcription factor that associates with the p53 tumor suppression pathway. CARF cooperates, co-localizes and is co-regulated with ARF, an ADP-ribosylation factor, and, through this interaction, helps to mediate ARF-p53-induced apoptotic signaling. This apoptotic pathway is implicated in cell cycle control, proper cellular development, response to DNA damage and the aging process, suggesting that CARF participates in various events throughout the cell. Mutations in the gene encoding CARF may be implicated in familial amyotrophic lateral sclerosis 2, a fatal neurodegenerative disease that is characterized by upper and lower motor neuron damage. Two isoforms of CARF exist due to alternative splicing events.

REFERENCES

1. Hadano, S., et al. 2001. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* 29: 166-173.
2. Hasan, M.K., et al. 2002. CARF is a novel protein that cooperates with mouse p19ARF (human p14ARF) in activating p53. *J. Biol. Chem.* 277: 37765-37770.
3. Tao, X., et al. 2002. A calcium-responsive transcription factor, CARF, that regulates neuronal activity-dependent expression of BDNF. *Neuron* 33: 383-395.

CHROMOSOMAL LOCATION

Genetic locus: CARF (human) mapping to 2q33.2.

PRODUCT

CARF 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CARF shRNA Plasmid (h): sc-72795-SH and CARF shRNA (h) Lentiviral Particles: sc-72795-V as alternate gene silencing products.

For independent verification of CARF (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-72795A, sc-72795B and sc-72795C.

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

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

CARF (14.4): sc-101206 is recommended as a control antibody for monitoring of CARF 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 CARF gene expression knockdown using RT-PCR Primer: CARF (h)-PR: sc-72795-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.

PROTOCOLS

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