

CA I siRNA (h): sc-60307

BACKGROUND

Carbonic anhydrases (CAs), also designated carbonate dehydratases or carbonate hydrolyases, form a large family of genes that encode zinc metalloenzymes of great physiologic importance. As catalysts of the reversible hydration of carbon dioxide, these enzymes participate in a variety of biologic processes, including respiration, acid-base balance, bone resorption and calcification as well as the formation of aqueous humor, cerebrospinal fluid, saliva and gastric acid. Genes in the α -carbonic anhydrase family encode either active carbonic anhydrase isozymes or "acatalytic" (devoid of CO₂ hydration activity) carbonic anhydrase-related proteins. Human CA I (CA1) is encoded by the CA1 gene, which maps to a region on chromosome 8q21.2 that harbors a cluster of CA genes. CA I localizes to the cytoplasm and research indicates that a severe deficiency of CA I does not result in any obvious hematological or renal consequences.

REFERENCES

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- Edwards, Y.H., et al. 1988. Assignment of the gene determining human carbonic anhydrase, CA I, to chromosome 8. *Ann. Hum. Genet.* 50: 123-129.
- Hewett-Emmett, D. and Tashian, R.E. 1996. Functional diversity, conservation and convergence in the evolution of the α -, β - and γ -carbonic anhydrase gene families. *Mol. Phylogenet. Evol.* 5: 50-77.
- Ichihara, N., et al. 1998. Immunohistolocalization of carbonic anhydrase isozymes (CA I, CA II and CA III) in bovine male reproductive tracts. *Okajimas Folia Anat. Jpn.* 74: 193-198.
- Supuran, C.T., et al. 2000. Carbonic anhydrase inhibitors: synthesis of sulfonamides incorporating 2,4,6-trisubstituted-pyridinium-ethylcarboxamido moieties possessing membrane-impermeability and *in vivo* selectivity for the membrane-bound (CA IV) versus the cytosolic (CA I and CA II) isozymes. *J. Enzyme Inhib.* 15: 381-401.

CHROMOSOMAL LOCATION

Genetic locus: CA1 (human) mapping to 8q21.2.

PRODUCT

CA I 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 CA I shRNA Plasmid (h): sc-60307-SH and CA I shRNA (h) Lentiviral Particles: sc-60307-V as alternate gene silencing products.

For independent verification of CA I (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-60307A, sc-60307B and sc-60307C.

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

CA I siRNA (h) is recommended for the inhibition of CA I 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

CA I (F-5): sc-393490 is recommended as a control antibody for monitoring of CA I 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 CA I gene expression knockdown using RT-PCR Primer: CA I (h)-PR: sc-60307-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.