

CAR siRNA (m): sc-39919

BACKGROUND

The coxsackie and adenovirus receptor (CAR) mediates viral infection by the binding of various adenoviruses through specific protein interactions. There is a high affinity between the viral knob domain and the extracellular amino-terminal domain (designated D1) of CAR. The D1 domain alone is sufficient for knob binding in transfected cells. Determining the specific interactions between CAR and adenoviruses is imperative in order to further develop gene therapy using viral hosts. CAR is expressed in many human and murine cell types. However, cells that express CAR at low levels are not efficiently infected by adenoviruses. Possible methods of avoiding this problem in certain cell types are by either supplementing CAR or modifying the Ad knob to bind to other receptors.

REFERENCES

1. Dmitriev, I., et al. 1998. An adenovirus vector with genetically modified fibers demonstrates expanded tropism via utilization of a coxsackievirus and adenovirus receptor-independent cell entry mechanism. *J. Virol.* 72: 9706-9713
2. Roelvink, P.W., et al. 1999. Identification of a conserved receptor-binding site on the fiber proteins of CAR-recognizing adenoviridae. *Science* 286: 1568-1571.
3. Bewley, M.C., et al. 1999. Structural analysis of the mechanism of adenovirus binding to its human cellular receptor, CAR. *Science* 286: 1579-1583.
4. Freimuth, P., et al. 1999. Coxsackievirus and adenovirus receptor amino-terminal immunoglobulin V-related domain binds adenovirus type 2 and fiber knob from adenovirus type 12. *J. Virol.* 73: 1392-1398.
5. Hidaka, C., et al. 1999. CAR-dependent and CAR-independent pathways of adenovirus vector-mediated gene transfer and expression in human fibroblasts. *J. Clin. Invest.* 103: 579-587.
6. Kirby, I., et al. 2000. Identification of contact residues and definition of the CAR-binding site of adenovirus type 5 fiber protein. *J. Virol.* 74: 2804-2813.

CHROMOSOMAL LOCATION

Genetic locus: Cxadr (mouse) mapping to 16 C3.1.

PRODUCT

CAR siRNA (m) 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 CAR shRNA Plasmid (m): sc-39919-SH and CAR shRNA (m) Lentiviral Particles: sc-39919-V as alternate gene silencing products.

For independent verification of CAR (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-39919A, sc-39919B and sc-39919C.

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

CAR siRNA (m) is recommended for the inhibition of CAR expression in mouse 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

CAR (E-1): sc-373791 is recommended as a control antibody for monitoring of CAR 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 CAR gene expression knockdown using RT-PCR Primer: CAR (m)-PR: sc-39919-PR (20 μ l, 525 bp). 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.