

vinculin siRNA (m): sc-36819

BACKGROUND

Focal adhesions are identified as areas within the plasma membrane of tissue culture cells that adhere tightly to the underlying substrate. *In vivo*, these regions are involved in the adhesion of cells to the extracellular matrix. Paxillin and vinculin are cytoskeletal, focal adhesion proteins that are components of a protein complex which links the Actin network to the plasma membrane. Vinculin binding sites have been identified on other cytoskeletal proteins, including Talin and α -actinin. In addition, vinculin, Talin and α -actinin each contain Actin binding sites. Expression of vinculin and Talin have been shown to be affected by the level of Actin expression. α -Actinin has been shown to link Actin to integrins in the plasma membrane through interactions with the vinculin and Talin complex or by a direct interaction with integrin.

REFERENCES

1. Burridge, K., et al. 1988. Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* 4: 487-525.
2. Gilmore, A.P., et al. 1992. Further characterization of the Talin-binding site in the cytoskeletal protein vinculin. *J. Cell Sci.* 103: 719-731.
3. Wood, C.K., et al. 1994. Characterisation of the paxillin-binding site and the C-terminal focal adhesion targeting sequence in vinculin. *J. Cell Sci.* 107: 709-717.
4. Gluck, U., et al. 1994. Modulation of α -actinin levels affects cell motility and confers tumorigenicity on 3T3 cells. *J. Cell Sci.* 107: 1773-1782.
5. Schevzov, G., et al. 1995. Impact of Actin gene expression on vinculin, Talin, cell spreading, and motility. *DNA Cell Biol.* 14: 689-700.
6. Gilmore, A.P., et al. 1996. Regulation of vinculin binding to Talin and Actin by phosphatidylinositol-4-5-bisphosphate. *Nature* 381: 531-535.
7. Hemmings, L., et al. 1996. Talin contains three Actin-binding sites each of which is adjacent to a vinculin-binding site. *J. Cell Sci.* 109: 2715-2726.

CHROMOSOMAL LOCATION

Genetic locus: Vcl (mouse) mapping to 14 A3.

PRODUCT

vinculin 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 vinculin shRNA Plasmid (m): sc-36819-SH and vinculin shRNA (m) Lentiviral Particles: sc-36819-V as alternate gene silencing products.

For independent verification of vinculin (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-36819A, sc-36819B and sc-36819C.

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

vinculin siRNA (m) is recommended for the inhibition of vinculin 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

vinculin (7F9): sc-73614 is recommended as a control antibody for monitoring of vinculin 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 vinculin gene expression knockdown using RT-PCR Primer: vinculin (m)-PR: sc-36819-PR (20 μ l, 549 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.