

hamartin siRNA (r): sc-270022

BACKGROUND

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by mental retardation and the widespread development of distinctive tumors termed hamartomas. Two different genetic loci have been linked to TSC; one of these loci, the tuberous sclerosis-2 gene (TSC2), encodes a protein called tuberin and the other loci, tuberous sclerosis-1 gene (TSC1), encodes a protein called hamartin. Tuberin and hamartin interact with each other forming a cytoplasmic complex. Hamartin interacts with the ezrin-radixin-moesin (ERM) family of Actin-binding proteins and inhibition of hamartin activity results in loss of cell adhesion. Hamartin is present in most adult tissues with strong expression in brain, heart, and kidney.

REFERENCES

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2. van Slegtenhorst, M., et al. 1997. Identification of the tuberous sclerosis gene TSC1 on chromosome 9q34. *Science* 277: 805-808.
3. Young, J. and Povey, S. 1998. The genetic basis of tuberous sclerosis. *Mol. Med. Today* 4: 313-319.
4. Plank, T.L., et al. 1998. Hamartin, the product of the tuberous sclerosis 1 (TSC1) gene, interacts with tuberin and appears to be localized to cytoplasmic vesicles. *Cancer Res.* 58: 4766-4770.
5. Nellist, M., et al. 1999. Characterization of the cytosolic tuberin-hamartin complex. Tuberin is a cytosolic chaperone for hamartin. *J. Biol. Chem.* 274: 35647-35652.
6. Plank, T.L., et al. 1999. The expression of hamartin, the product of the TSC1 gene, in normal human tissues and in TSC1- and TSC2-linked angiomyolipomas. *Mod. Pathol.* 12: 539-545.
7. Fukuhara, S., et al. 2000. A new twist for the tumour suppressor hamartin. *Nat. Cell Biol.* 2: E76-E78.
8. Lamb, R.F., et al. 2000. The TSC1 tumour suppressor hamartin regulates cell adhesion through ERM proteins and the GTPase Rho. *Nat. Cell Biol.* 2: 281-287.

CHROMOSOMAL LOCATION

Genetic locus: Tsc1 (rat) mapping to 3p12.

PRODUCT

hamartin siRNA (r) 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 hamartin shRNA Plasmid (r): sc-270022-SH and hamartin shRNA (r) Lentiviral Particles: sc-270022-V as alternate gene silencing products.

For independent verification of hamartin (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270022A, sc-270022B and sc-270022C.

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

hamartin siRNA (r) is recommended for the inhibition of hamartin expression in rat 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

hamartin (C-8): sc-377386 is recommended as a control antibody for monitoring of hamartin 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 hamartin gene expression knockdown using RT-PCR Primer: hamartin (r)-PR: sc-270022-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.