

fumarate hydratase siRNA (h): sc-105377

BACKGROUND

Fumarate hydratase, a ubiquitously expressed mitochondrial enzyme, catalyzes the reversible hydration of fumaric acid to yield L-malic acid during the Krebs cycle. Germline mutations in the fumarate hydratase gene cause a predisposition to renal defects such as multiple cutaneous and uterine leiomyoma (MCL) syndrome. Furthermore, mutations also correlate with renal and smooth muscle tumors, but not with prostate cancer. Additionally, like other metabolic diseases, fumarate hydratase deficiency correlates with seizures, due to prenatal brain dysgenesis.

REFERENCES

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2. Eng, C., et al. 2003. A role for mitochondrial enzymes in inherited neoplasia and beyond. *Nat. Rev. Cancer* 3: 193-202.
3. Martinez-Mir, A., et al. 2003. Germline fumarate hydratase mutations in families with multiple cutaneous and uterine leiomyomata. *J. Invest. Dermatol.* 121: 741-744.
4. Bevan, S., et al. 2003. Germline mutations in fumarate hydratase (FH) do not predispose to prostate cancer. *Prostate Cancer Prostatic Dis.* 6: 12-14.
5. Pollard, P.J., et al. 2003. The TCA cycle and tumorigenesis: the examples of fumarate hydratase and succinate dehydrogenase. *Ann. Med.* 35: 632-639.
6. McGrath, J.A. 2003. Biologic lessons from mutations in the Krebs cycle enzyme, fumarate hydratase. *J. Invest. Dermatol.* 121: vii.
7. Gross, K.L., et al. 2004. Involvement of fumarate hydratase in nonsyndromic uterine leiomyomas: genetic linkage analysis and FISH studies. *Genes Chromosomes Cancer* 41: 183-190.
8. Lehtonen, R., et al. 2004. Biallelic inactivation of fumarate hydratase (FH) occurs in nonsyndromic uterine leiomyomas but is rare in other tumors. *Am. J. Pathol.* 164: 17-22.

CHROMOSOMAL LOCATION

Genetic locus: FH (human) mapping to 1q43.

PRODUCT

fumarate hydratase 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 fumarate hydratase shRNA Plasmid (h): sc-105377-SH and fumarate hydratase shRNA (h) Lentiviral Particles: sc-105377-V as alternate gene silencing products.

For independent verification of fumarate hydratase (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-105377A, sc-105377B and sc-105377C.

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

fumarate hydratase siRNA (h) is recommended for the inhibition of fumarate hydratase 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

fumarate hydratase (H-6): sc-393992 is recommended as a control antibody for monitoring of fumarate hydratase 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 fumarate hydratase gene expression knockdown using RT-PCR Primer: fumarate hydratase (h)-PR: sc-105377-PR (20 μ l, 479 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.

PROTOCOLS

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