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TRP2 siRNA (m): sc-41662

BACKGROUND

Tyrosinase (TYR), a type I membrane protein and copper-containing enzyme, is involved in the production of melanin, the primary pigment found in vertebrates. Melanin biogenesis requires the enzymatic activity of TYR, which catalyzes the critical and rate-limiting step of tyrosine hydroxylation in the biosynthesis of melanin. Defects effecting TYR activity result in various forms of albinism. The TYR-related proteins, TRP1 and TRP2, are also specifically expressed in melanocytes, and they likewise contribute to the synthesis of melanin within the melanosomes. The TRPs, including TYR, all share a similar transmembrane region, contain two metal-binding regions and a cysteine-rich epidermal growth factor motif and are localized in the melanosomal membrane. These proteins, however, have distinct catalytic activity, and they individually contribute to the biosynthesis of melanin biopolymers. The TRPs are believed to exist as a multi-enzyme complex, as these proteins form aggregates together, and the expression of TRP1 also helps stabilize TYR in melanocytes.

REFERENCES

1. Korner, A., et al. 1982. Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. *Science* 217: 1163-1165.
2. Shibahara, S., et al. 1986. Cloning and expression of cDNA encoding mouse tyrosinase. *Nucleic Acids Res.* 14: 2413-2427.
3. Hearing, V.J., et al. 1987. Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation. *Int. J. Biochem.* 19: 1141-1147.
4. Tripathi, R.K., et al. 1992. Mutational mapping of the catalytic activities of human tyrosinase. *J. Biol. Chem.* 267: 23707-23712.
5. Tsukamoto, K., et al. 1992. A second tyrosinase-related protein, TRP-2, is a melanogenic enzyme termed DOPochrome tautomerase. *EMBO J.* 11: 519-526.
6. Bouchard, B., et al. 1994. Molecular characterization of a human tyrosinase-related-protein-2 cDNA. Patterns of expression in melanocytic cells. *Eur. J. Biochem.* 219: 127-134.
7. Orlow, S.J., et al. 1994. High-molecular-weight forms of tyrosinase and the tyrosinase-related proteins: evidence for a melanogenic complex. *J. Invest. Dermatol.* 103: 196-201.

CHROMOSOMAL LOCATION

Genetic locus: Dct (mouse) mapping to 14 E4.

PRODUCT

TRP2 siRNA (m) is a pool of 2 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 TRP2 shRNA Plasmid (m): sc-41662-SH and TRP2 shRNA (m) Lentiviral Particles: sc-41662-V as alternate gene silencing products.

For independent verification of TRP2 (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-41662A and sc-41662B.

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

TRP2 siRNA (m) is recommended for the inhibition of TRP2 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

TRP2 (E-10): sc-166716 is recommended as a control antibody for monitoring of TRP2 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 TRP2 gene expression knockdown using RT-PCR Primer: TRP2 (m)-PR: sc-41662-PR (20 μ l, 578 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.