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Paraxis siRNA (h): sc-45841

BACKGROUND

The novel basic helix-loop-helix (bHLH) transcription factor, twist, is a putative regulator of mesodermal differentiation and myogenesis. Twist is expressed throughout the epithelial somite but not in the myotome. Twist requires dimerization with E proteins, such as paraxis, and inhibits myogenic regulatory factors. As an early transcriptional regulator, paraxis determines the mesoderm pattern and governs the type of mesoderm-derived cells. Paraxis is also involved in the regulation of morphogenetic activities during somitogenesis. A nuclear protein containing one bHLH domain, Paraxis requires dimerization with another protein in order to bind DNA efficiently.

REFERENCES

1. Carpio, R., et al. 2004. *Xenopus* paraxis homolog shows novel domains of expression. *Dev. Dyn.* 231: 609-613.
2. Wilson-Rawls, J., et al. 2004. Paraxis is a basic helix-loop-helix protein that positively regulates transcription through binding to specific E-box elements. *J. Biol. Chem.* 279: 37685-37692.
3. Nakaya, Y., et al. 2004. Mesenchymal-epithelial transition during somitic segmentation is regulated by differential roles of Cdc42 and Rac1. *Dev. Cell* 7: 425-438.
4. Borue, X., et al. 2004. Normal and aberrant craniofacial myogenesis by grafted trunk somitic and segmental plate mesoderm. *Development* 131: 3967-3980.
5. Wilm, B., et al. 2004. The forkhead genes, Foxc1 and Foxc2, regulate paraxial versus intermediate mesoderm cell fate. *Dev. Biol.* 271: 176-189.
6. Schmidt, C., et al. 2004. Wnt 6 regulates the epithelialisation process of the segmental plate mesoderm leading to somite formation. *Dev. Biol.* 271: 198-209.

CHROMOSOMAL LOCATION

Genetic locus: TCF15 (human) mapping to 20p13.

PRODUCT

Paraxis 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 Paraxis shRNA Plasmid (h): sc-45841-SH and Paraxis shRNA (h) Lentiviral Particles: sc-45841-V as alternate gene silencing products.

For independent verification of Paraxis (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-45841A, sc-45841B and sc-45841C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

Paraxis siRNA (h) is recommended for the inhibition of Paraxis 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

Paraxis (H-55): sc-98796 is recommended as a control antibody for monitoring of Paraxis 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Paraxis gene expression knockdown using RT-PCR Primer: Paraxis (h)-PR: sc-45841-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.