

twist siRNA (h): sc-38604

BACKGROUND

Members of the myogenic determination family are basic helix-loop-helix (bHLH) proteins that can be separated into two classes. Class A proteins include the ubiquitously expressed E-box binding factors E12/E47, ITF2 and HEB (BETA1 or HTF4). Class B proteins such as MyoD, myogenin and NeuroD (BETA2) are transiently expressed and exhibit a much more limited tissue distribution. Class A proteins heterodimerize with class B proteins to activate DNA transcription. Working in opposition to these positively acting factors are a specialized group of proteins that function as dominant negative regulators. Muscle tissue is derived from a subset of cells originating from the embryonic mesoderm. 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 the E proteins and inhibits myogenic regulatory factors. It has been implicated as regulator of the temporal and spatial formation of myotomes.

REFERENCES

1. Lee, J.E., et al. 1995. Conversion of *Xenopus* ectoderm into neurons by neuroD, a basic helix-loop-helix protein. *Science* 268: 836-844.
2. Naya, F.J., et al. 1995. Tissue-specific regulation of the Insulin gene by a novel basic helix-loop-helix transcription factor. *Genes Dev.* 9: 1009-1019.

CHROMOSOMAL LOCATION

Genetic locus: TWIST1 (human) mapping to 7p21.1.

PRODUCT

twist 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 twist shRNA Plasmid (h): sc-38604-SH and twist shRNA (h) Lentiviral Particles: sc-38604-V as alternate gene silencing products.

For independent verification of twist (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-38604A, sc-38604B and sc-38604C.

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

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

twist (Twist2C1a): sc-81417 is recommended as a control antibody for monitoring of twist 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 twist gene expression knockdown using RT-PCR Primer: twist (h)-PR: sc-38604-PR (20 μ l, 509 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Velpula, K.K., et al. 2011. Cord blood stem cells revert glioma stem cell EMT by down regulating transcriptional activation of Sox2 and twist1. *Oncotarget* 2: 1028-1042.
2. Pallier, K., et al. 2012. Twist1 a new determinant of epithelial to mesenchymal transition in EGFR mutated lung adenocarcinoma. *PLoS ONE* 7: e29954.
3. Jin, H.O., et al. 2012. Silencing of twist1 sensitizes NSCLC cells to cisplatin via AMPK-activated mTOR inhibition. *Cell Death Dis.* 3: e319.
4. Sossey-Alaoui, K., et al. 2014. Kindlin-3 enhances breast cancer progression and metastasis by activating twist-mediated angiogenesis. *FASEB J.* 28: 2260-2271.
5. Avasarala, S., et al. 2015. PRMT1 is a novel regulator of epithelial-mesenchymal-transition in non-small cell lung cancer. *J. Biol. Chem.* 290: 13479-13489.

RESEARCH USE

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