

Ang-2 siRNA (h): sc-39305

BACKGROUND

Tie-1 and Tie-2 (also designated Tek) are novel cell surface receptor tyrosine kinases. The extracellular domain of Tie-1 has an unusual multidomain structure consisting of a cluster of three epidermal growth factor homology motifs localized between two immunoglobulin-like loops, which are followed by three Fibronectin type III repeats next to the transmembrane region. Angiopoietin-1 (Ang-1) is a secreted ligand for Tie-2. Preliminary biochemical analyses of Ang-1 reveal a potential Fibrinogen-like domain at the carboxy-terminus and coiled-coil regions in the amino-terminus. Ang-1 is an angiogenic factor that is thought to be involved in endothelial development. A related protein, angiopoietin-2 (Ang-2), has been identified as a naturally occurring antagonist of Ang-1 activation of Tie-2. In adult tissue, Ang-2 expression seems to be restricted to sites of vascular remodeling.

REFERENCES

1. Partanen, J., et al. 1992. A novel endothelial cell surface receptor tyrosine kinase with extracellular epidermal growth factor homology domains. *Mol. Cell. Biol.* 12: 1698-1707.
2. Dumont, D.J., et al. 1992. Tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors. *Oncogene* 7: 1471-1480.
3. Sato, T.N., et al. 1993. Tie-1 and Tie-2 define another class of putative receptor tyrosine kinase genes expressed in early embryonic vascular system. *Proc. Natl. Acad. Sci. USA* 90: 9355-9358.
4. Dumont, D.J., et al. 1993. The endothelial-specific receptor tyrosine kinase, Tek, is a member of a new subfamily of receptors. *Oncogene* 8: 1293-1301.

CHROMOSOMAL LOCATION

Genetic locus: ANGPT2 (human) mapping to 8p23.1.

PRODUCT

Ang-2 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 Ang-2 shRNA Plasmid (h): sc-39305-SH and Ang-2 shRNA (h) Lentiviral Particles: sc-39305-V as alternate gene silencing products.

For independent verification of Ang-2 (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-39305A, sc-39305B and sc-39305C.

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

Ang-2 shRNA (h) is recommended for the inhibition of Ang-2 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

Ang-2 (F-1): sc-74403 is recommended as a control antibody for monitoring of Ang-2 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 Ang-2 gene expression knockdown using RT-PCR Primer: Ang-2 (h)-PR: sc-39305-PR (20 μ l, 521 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Holopainen, T., et al. 2012. Effects of angiopoietin-2-blocking antibody on endothelial cell-cell junctions and lung metastasis. *J. Natl. Cancer Inst.* 104: 461-475.

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.