

EphA5 siRNA (h): sc-39938

BACKGROUND

The Eph subfamily represents the largest group of receptor protein tyrosine kinases identified to date. While the biological activities of these receptors have yet to be determined, there is increasing evidence that they are involved in central nervous system function and in development. The Eph subfamily receptors of human origin (and their murine/avian homologs) include EphA1 (Eph), EphA2 (Eck), EphA3 (Hek4), EphA4 (Hek8), EphA5 (Hek7), EphA6 (Hek12), EphA7 (Hek11/MDK1), EphA8 (Hek3), EphB1 (Hek6), EphB2 (Hek5), EphB3 (Cek10, Hek2), EphB4 (Htk), EphB5 (Hek9) and EphB6 (Mep). Ligands for Eph receptors include ephrin-A4 (LERK-4) which binds EphA3 and EphB1. ephrin-A2 (ELF-1) has been described as the ligand for EphA4, ephrin-A3 (Ehk1-L) as the ligand for EphA5 and ephrin-B2 (Htk-L) as the ligand for EphB4 (Htk).

REFERENCES

1. Beckmann, M.P., et al. 1994. Molecular characterization of a family of ligands for Eph-related tyrosine kinase receptors. *EMBO J.* 13: 3757-3762.
2. Cheng, H.J., et al. 1994. Identification and cloning of ELF-1, a developmentally expressed ligand for the Mek4 and Sek receptor tyrosine kinases. *Cell* 79: 157-168.
3. Ciossek, T., et al. 1995. Identification of alternatively spliced mRNAs encoding variants of MDK1, a novel receptor tyrosine kinase expressed in the murine nervous system. *Oncogene* 10: 97-108.
4. Kozlosky, C.J., et al. 1995. Ligands for the receptor tyrosine kinases Hek and Elk: isolation of cDNAs encoding a family of proteins. *Oncogene* 10: 299-306.
5. Fox, G.M., et al. 1995. DNA cloning and tissue distribution of five human Eph-like receptor protein-tyrosine kinases. *Oncogene* 10: 897-905.
6. Valenzuela, D.M., et al. 1995. Identification of full length and truncated forms of Ehk3, a novel member of the Eph receptor tyrosine kinase family. *Oncogene* 10: 1573-1580.
7. Bennett, B.D., et al. 1995. Molecular cloning of a ligand for the Eph-related receptor protein-tyrosine kinase Htk. *Proc. Natl. Acad. Sci. USA* 92: 1866-1870.

CHROMOSOMAL LOCATION

Genetic locus: EPHA5 (human) mapping to 4q13.1.

PRODUCT

EphA5 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 EphA5 shRNA Plasmid (h): sc-39938-SH and EphA5 shRNA (h) Lentiviral Particles: sc-39938-V as alternate gene silencing products.

For independent verification of EphA5 (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-39938A, sc-39938B and sc-39938C.

PROTOCOLS

See our web site at www.scbt.com 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

EphA5 siRNA (h) is recommended for the inhibition of EphA5 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

EphA5 (8B10B1): sc-517242 is recommended as a control antibody for monitoring of EphA5 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 EphA5 gene expression knockdown using RT-PCR Primer: EphA5 (h)-PR: sc-39938-PR (20 μ l). 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.