

Amphiregulin siRNA (m): sc-39413

BACKGROUND

Epidermal growth factor (EGF) family member Amphiregulin was initially characterized as a schwannoma-derived growth factor (SDGF) that was expressed in response to androgen in the SC2G murine cell line. Amphiregulin has subsequently been characterized as an important growth factor for normal human keratinocyte proliferation. Amphiregulin is produced and secreted by keratinocytes and acts as an autocrine growth factor. Amphiregulin binds ErbB-1, which is essential for epithelial development in the skin, lung and gastrointestinal tract. Withdrawal of Amphiregulin has been shown to result in down regulation of telomerase activity in human keratinocytes and this suggests that Amphiregulin plays a role in cell senescence.

REFERENCES

1. Cook, P.W., et al. 1991. A heparin sulfate-regulated human keratinocyte autocrine factor is similar or identical to Amphiregulin. *Mol. Cell. Biol.* 11: 2547-2557.
2. Sonoda, H., et al. 1992. Androgen-responsive expression and mitogenic activity of schwannoma-derived growth factor on an androgen-dependent Shionogi mouse mammary carcinoma cell line. *Biochem. Biophys. Res. Commun.* 185: 103-109.
3. Elenius, K., et al. 1997. Activation of HER4 by heparin-binding EGF-like growth factor stimulates chemotaxis but not proliferation. *EMBO J.* 16: 1268-1278.
4. Shirakata, Y., et al. 2000. Epiregulin, a novel member of the epidermal growth factor family, is an autocrine growth factor in normal human keratinocytes. *J. Biol. Chem.* 275: 5748-5753.
5. Matsui, M., et al. 2000. Influence of aging and cell senescence on telomerase activity in keratinocytes. *J. Dermatol. Sci.* 22: 80-87.

CHROMOSOMAL LOCATION

Genetic locus: Areg (mouse) mapping to 5 E1.

PRODUCT

Amphiregulin siRNA (m) 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 Amphiregulin shRNA Plasmid (m): sc-39413-SH and Amphiregulin shRNA (m) Lentiviral Particles: sc-39413-V as alternate gene silencing products.

For independent verification of Amphiregulin (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-39413A, sc-39413B and sc-39413C.

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

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

Amphiregulin (G-4): sc-74501 is recommended as a control antibody for monitoring of Amphiregulin 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 Amphiregulin gene expression knockdown using RT-PCR Primer: Amphiregulin (m)-PR: sc-39413-PR (20 μ l, 425 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.