SANTA CRUZ BIOTECHNOLOGY, INC.

GCP-2 siRNA (h): sc-39350

BACKGROUND

Human granulocyte chemotactic protein 2 (GCP-2) is a member of the ELR-expressing C-X-C subfamily of chemokines (ELR+-CXC) and acts as a potent chemoattractant of neutrophils in the course of acute inflammation. GCP-2 is highly produced by MG-63 osteosarcoma cells and induces neovascularization, suggesting that it may be involved in tumor development and metastasis formation. ELR+-CXC chemokines and their receptors provide multiple levels of regulation for chemokine-specific control of inflammatory processes. GCP-2 is the only ELR+-CXC chemokine, except for IL-8, that is an effective ligand for CXCR-1 and CXCR-2. CXCR-1 and CXCR-2 belong to a superfamily of G protein-coupled receptors (GPCR), whose signaling is mediated by their coupling to heterotrimeric G proteins, resulting in the exchange of GDP for GTP on the subunit of the G protein.

REFERENCES

1. Savarese, T.M., et al. 1992. *In vitro* mutagenesis and the search for structure-function relationship among G protein-coupled receptors. Biochem. J. 283: 1-19.
2. Probst, W.C., et al. 1992. Sequence alignment of the G protein-coupled receptor superfamily. DNA Cell Biol. 11: 1-20.
3. Ben-Baruch, A., et al. 1995. Signals and receptors involved in recruitment of inflammatory cells. J. Biol. Chem. 270: 703-706.
4. Wess, J. 1997. G protein-coupled receptors: molecular mechanisms involved in receptor activation and selectivity of G protein recognition. FASEB J. 11: 346-354.
5. Wuyts, A., et al. 1997. Characterization of synthetic human granulocyte chemotactic protein 2: usage of chemokine receptors CXCR-1 and CXCR-2 and *in vivo* inflammatory properties. Biochemistry 36: 2716-2723.
6. Van Damme, J., et al. 1997. Granulocyte chemotactic protein-2 and related C-X-C chemokines: from gene regulation to receptor usage. J. Leukoc. Biol. 62: 563-569.
7. Feniger-Barish, R., et al. 2000. GCP-2-induced internalization of IL-8 receptors: hierarchical relationships between GCP-2 and other ELR+-CXC chemokines and mechanisms regulating CXCR-2 internalization and recycling. Blood 95: 1551-1559.

CHROMOSOMAL LOCATION

Genetic locus: CXCL6 (human) mapping to 4q13.3.

PRODUCT

GCP-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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GCP-2 shRNA Plasmid (h): sc-39350-SH and GCP-2 shRNA (h) Lentiviral Particles: sc-39350-V as alternate gene silencing products.

For independent verification of GCP-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-39350A, sc-39350B and sc-39350C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GCP-2 siRNA (h) is recommended for the inhibition of GCP-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 µM in 66 µl. 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

ENA-78 (D-6): sc-377026 is recommended as a control antibody for monitoring of GCP-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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 GCP-2 gene expression knockdown using RT-PCR Primer: GCP-2 (h)-PR: sc-39350-PR (20 µl). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.