SANTA CRUZ BIOTECHNOLOGY, INC.

GFRP siRNA (m): sc-145387

BACKGROUND

GTP cyclohydrolase I feedback regulatory protein (GFRP) is encoded by the gene GCHFR. GFRP mediates feedback inhibition of GTP cyclohydrolase I activity by tetrahydrobiopterin. GFRP also acts as a mediator for the stimulatory effect of phenylalanine on enzyme activity. L-phenylalanine reverses this inhibition. Cross-linking experiments have shown that GFRP is usually expressed as a homodimer or pentamer.

REFERENCES

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2. Milstien, S., Jaffe, H., Kowlessur, D. and Bonner, T.I. 1996. Purification and cloning of the GTP cyclohydrolase I feedback regulatory protein, GFRP. J. Biol. Chem. 271: 19743-19751.
3. Yoneyama, T., Brewer, J.M. and Hatakeyama, K. 1997. GTP cyclohydrolase I feedback regulatory protein is a pentamer of identical subunits. Purification, cDNA cloning, and bacterial expression. J. Biol. Chem. 272: 9690-9696.
4. Bader, G., Schiffmann, S., Herrmann, A., Fischer, M., Gütlich, M., Auerbach, G., Ploom, T., Bacher, A., Huber, R. and Lemm, T. 2001. Crystal structure of rat GTP cyclohydrolase I feedback regulatory protein, GFRP. J. Mol. Biol. 312: 1051-1057.
5. Maita, N., Hatakeyama, K., Okada, K. and Hakoshima, T. 2004. Structural basis of biopterin-induced inhibition of GTP cyclohydrolase I by GFRP, its feedback regulatory protein. J. Biol. Chem. 279: 51534-51540.
6. SWISS-PROT/TrEMBL (P30047). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html
7. http://harvester.embl.de/harvester/P300/P30047.htm

CHROMOSOMAL LOCATION

Genetic locus: Gchfr (mouse) mapping to 2 E5.

PRODUCT

GFRP siRNA (m) is a target-specific 19-25 nt siRNA 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 GFRP shRNA Plasmid (m): sc-145387-SH and GFRP shRNA (m) Lentiviral Particles: sc-145387-V as alternate gene silencing products.

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.

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

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

GFRP (D-11): sc-514098 is recommended as a control antibody for monitoring of GFRP 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 GFRP gene expression knockdown using RT-PCR Primer: GFRP (m)-PR: sc-145387-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.