SANTA CRUZ BIOTECHNOLOGY, INC.

DHFR siRNA (C. griseus): sc-270574

BACKGROUND

Dihydrofolate reductase (DHFR) catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, and is a crucial enzyme for the synthesis of purines, pyrimidines and some amino acids. Inhibition of the activity of this enzyme leads to arrest of DNA synthesis and cell death. Gene expression of methotrexate (MTX)-resistant variants of DHFR in normal hematopoietic cells is a potential strategy to permit administration of larger doses of MTX by alleviating drug toxicity in normal cells and tissues that are drug sensitive.

REFERENCES

1. Walker, V.K., Tyshenko, M.G., Kuiper, M.J., Dargar, R.V., Yuhas, D.A., Cruickshank, P.A. and Chaguturu, R. 2000. Tobacco budworm dihydrofolate reductase is a promising target for insecticide discovery. Eur. J. Biochem. 267: 394-403.
2. Li, R., Sirawaraporn, R., Chitnumsub, P., Sirawaraporn, W., Wooden, J., Athappilly, F., Turley, S. and Hol, W.G. 2000. Three-dimensional structure of *M. tuberculosis* dihydrofolate reductase reveals opportunities for the design of novel tuberculosis drugs. J. Mol. Biol. 295: 307-323.
3. Yoshikawa, T., Nakanishi, F., Ogura, Y., Oi, D., Omasa, T., Katakura, Y., Kishimoto, M. and Suga, K. 2000. Amplified gene location in chromosomal DNA affected recombinant protein production and stability of amplified genes. Biotechnol. Prog. 16: 710-715.
4. Belur, L.R., Boelk-Galvan, D., Diers, M.D., McIvor, R.S. and Zimmerman, C.L. 2001. Methotrexate accumulates to similar levels in animals transplanted with normal versus drug-resistant transgenic marrow. Cancer Res. 61: 1522-1526.
5. Grillari, J., Fortschegger, K., Grabherr, R.M., Hohenwarter, O., Kunert, R. and Katinger, H. 2001. Analysis of alterations in gene expression after amplification of recombinantgenes in CHO cells. J. Biotechnol. 87: 59-65.

CHROMOSOMAL LOCATION

Genetic locus: Dhfr *(C. griseus)* mapping to 2.

PRODUCT

DHFR siRNA (C. griseus) 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 DHFR shRNA Plasmid (C. griseus): sc-270574-SH and DHFR shRNA (C. griseus) Lentiviral Particles: sc-270574-V as alternate gene silencing products.

For independent verification of DHFR (C. griseus) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270574A, sc-270574B and sc-270574C.

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

DHFR siRNA (C. griseus) is recommended for the inhibition of DHFR expression in *C. griseus* 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DHFR gene expression knockdown using RT-PCR Primer: DHFR (C. griseus)-PR: sc-270574-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.