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DPYD siRNA (m): sc-45327

BACKGROUND

Dihydropyrimidine dehydrogenase (DPYD) catalyzes the first rate-limiting step of the NADPH-dependent catabolism of uracil and thymine to dihydrouracil and dihydrothymine; thus, a deficiency of DPYD leads to an accumulation of uracil and thymine. Abnormal concentrations of these metabolites in bodily fluids may be the cause of neurological disease and a contraindication for treatment of cancer patients with certain pyrimidine analogs. DPYD also catalyzes the anticancer agent 5-fluorouracil (5-FU) pathway and is involved in the efficacy and toxicity of 5-FU. Variations in DPYD concentration may arise from alterations at the transcriptional level of the dihydropyrimidine dehydrogenase gene. Specifically, hypermethylation of the DPYD promoter downregulates dihydropyrimidine dehydrogenase expression. Deficient DPYD alleles may constitute a risk factor for severe toxicity following treatment with 5-FU.

REFERENCES

1. Godtel, R., et al. 1978. Puerperal psychoses. 38: 304-316.
2. Tuchman, M., et al. 1989. Dihydropyrimidine dehydrogenase activity in human blood mononuclear cells. *Enzyme*. 42: 15-24.
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5. Fischer, J., et al. 2003. Mutational analysis of the human dihydropyrimidine dehydrogenase gene by denaturing high-performance liquid chromatography. *Genet. Test.* 7: 97-105.
6. Enns, G.M., et al. 2004. Head imaging abnormalities in dihydropyrimidine dehydrogenase deficiency. *J. Inherit. Metab. Dis.* 27: 513-522.
7. Al-Sanna'a, N.A., et al. 2005. Dihydropyrimidine dehydrogenase deficiency presenting at birth. *J. Inherit. Metab. Dis.* 28: 793-796.

CHROMOSOMAL LOCATION

Genetic locus: *Dpyd* (mouse) mapping to 3 G1.

PRODUCT

DPYD 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 DPYD shRNA Plasmid (m): sc-45327-SH and DPYD shRNA (m) Lentiviral Particles: sc-45327-V as alternate gene silencing products.

For independent verification of DPYD (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-45327A, sc-45327B and sc-45327C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

DPYD (A-5): sc-376712 is recommended as a control antibody for monitoring of DPYD 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DPYD gene expression knockdown using RT-PCR Primer: DPYD (m)-PR: sc-45327-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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