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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

# DPYD shRNA (h) Lentiviral Particles: sc-45326-V

## 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.
3. van Gennip, A.H., et al. 1997. Inborn errors of pyrimidine degradation: clinical, biochemical and molecular aspects. *J. Inher. Metab. Dis.* 20: 203-213.
4. Johnson, M.R., et al. 1997. Semi-automated radioassay for determination of dihydropyrimidine dehydrogenase (DPD) activity. Screening cancer patients for DPD deficiency, a condition associated with 5-fluorouracil toxicity. *J. Chromatogr. B. Biomed. Sci. Appl.* 696: 183-191.
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.

## CHROMOSOMAL LOCATION

Genetic locus: DPYD (human) mapping to 1p21.3.

## PRODUCT

DPYD shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see DPYD siRNA (h): sc-45326 and DPYD shRNA Plasmid (h): sc-45326-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

DPYD shRNA (h) Lentiviral Particles is recommended for the inhibition of DPYD expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (h)-PR: sc-45326-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.