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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

www.szabo-scandic.com

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

LDH-B shRNA (h) Lentiviral Particles: sc-45899-V

BACKGROUND

The lactate dehydrogenase family (LDH) catalyzes the final step of anaerobic glycolysis, the conversion of L-lactate and NAD to pyruvate and NADH. The LDH family consists of three members, LDH-A, LDH-B and LDH-C, all of which form tetramers consisting of four subunits. However, each family member displays a specific tissue distribution pattern, with LDH-A and LDH-B predominant in several tissues, specifically LDH-A in muscle and LDH-B in heart, while LDH-C expression is confined to testis and sperm. LDHs function as powerful markers for germ cell tumors. The genes encoding human LDH-A and LDH-C map to chromosome 11, while the human LDH-B gene maps to chromosome 12. Deficiency in the LDH-A gene is linked to exertional myoglobinuria.

REFERENCES

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- Edwards, Y.H., et al. 1987. Locus determining the human sperm-specific lactate dehydrogenase, LDH-C, is syntenic with LDH-A. *Dev. Genet.* 8: 219-232.
- Takano, T. and Li, S.S. 1989. Human testicular lactate dehydrogenase-C gene is interrupted by six introns at positions homologous to those of LDH-A (muscle) and LDH-B (heart) genes. *Biochem. Biophys. Res. Commun.* 159: 579-583.
- LeVan, K.M. and Goldberg, E. 1991. Properties of human testis-specific lactate dehydrogenase expressed from *Escherichia coli*. *Biochem. J.* 273: 587-592.
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CHROMOSOMAL LOCATION

Genetic locus: LDHB (human) mapping to 12p12.1.

PRODUCT

LDH-B 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 μ l frozen stock containing 1.0×10^6 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 LDH-B siRNA (h): sc-45899 and LDH-B shRNA Plasmid (h): sc-45899-SH as alternate gene silencing products.

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.

APPLICATIONS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

LDH-B (S-19): sc-27234 is recommended as a control antibody for monitoring of LDH-B 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LDH-B gene expression knockdown using RT-PCR Primer: LDH-B (h)-PR: sc-45899-PR (20 μ l, 519 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

STORAGE

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

PROTOCOLS

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