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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) 



# MCT4 shRNA (h2) Lentiviral Particles: sc-45892-V

## BACKGROUND

Monocarboxylates, such as lactate and pyruvate, play an integral role in cellular metabolism. Lactic acid is produced in large quantities as a result of glycolysis, which provides the majority of ATP to cells under normal physiological conditions. However, accumulation of lactic acid leads to a decrease in intracellular pH and cessation of glycolysis. In order for glycolysis to continue at a high rate, lactic acid must be transported out of the cell. This transport process is carried out by a family of monocarboxylate transporters (MCTs), which function as proton symports and are stereoselective for L-lactate. The MCT family consists of at least eight members, MCT 1-8, which contain between 10-12 transmembrane-helical (TM) domains, with the amino- and carboxy-termini located in the cytoplasm. MCT1 is widely expressed and is the major form of MCT in tumor cells and erythrocytes. MCT2 is highly expressed in liver and testis, while MCT3 and MCT4 are predominantly expressed in skeletal muscle.

## REFERENCES

- Gerhart, D.Z., et al. 1997. Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats. *Am. J. Physiol.* 273: E207-213.
- Halestrap, A.P., et al. 1997. Lactate transport in heart in relation to myocardial ischemia. *Am. J. Cardiol.* 80: 17A-25A.
- Lin, R.Y., et al. 1998. Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J. Biol. Chem.* 273: 28959-28965.
- Price, N.T., et al. 1998. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* 329: 321-328.
- Halestrap, A.P., et al. 1999. The proton-linked monocarboxylate transporter (MCT) family: structure, function and regulation. *Biochem. J.* 343: 281-299.
- Juel, C., et al. 1999. Lactate transport in skeletal muscle—role and regulation of the monocarboxylate transporter. *J. Physiol.* 517: 633-642.

## CHROMOSOMAL LOCATION

Genetic locus: SLC16A3 (human) mapping to 17q25.3.

## PRODUCT

MCT4 shRNA (h2) 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 MCT4 siRNA (h2): sc-45892 and MCT4 shRNA Plasmid (h2): sc-45892-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

MCT4 shRNA (h2) Lentiviral Particles is recommended for the inhibition of MCT4 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

MCT4 (H-90): sc-50329 is recommended as a control antibody for monitoring of MCT4 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MCT4 gene expression knockdown using RT-PCR Primer: MCT4 (h2)-PR: sc-45892-PR (20  $\mu$ l, 482 bp). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## 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.

## PROTOCOLS

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