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- Mindermengenzuschlag
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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) 

COMT shRNA (h) Lentiviral Particles: sc-43693-V

BACKGROUND

Catechol-O-methyltransferase (COMT) plays a crucial role in the regulation of central dopaminergic systems by catalyzing the inactivation of catecholamines. It is widely distributed in most tissues in soluble and membrane-bound forms. COMT-mediated methylation metabolism of catecholamine neurotransmitters is a first-line detoxification pathway. A Val158Met polymorphism of the COMT gene affects activity of the enzyme and influences performance and efficiency of the prefrontal cortex of the brain. Sequential conversion of estradiol to methoxyestradiol by COMT, contributes to the antimitogenic effects of estradiol on vascular smooth muscle cell growth via estrogen receptor-independent mechanisms.

REFERENCES

- Masuda, M., et al. 2002. Assay of catechol-O-methyltransferase activity in human erythrocytes using norepinephrine as a natural substrate. *Ann. Clin. Biochem.* 39: 589-594.
- Inada, T., et al. 2003. Relationship between catechol-O-methyltransferase polymorphism and treatment-resistant schizophrenia. *Am. J. Med. Genet.* 120B: 35-39.
- Dubey, R.K., et al. 2004. Catecholamines block the antimitogenic effect of estradiol on human coronary artery smooth muscle cells. *J. Clin. Endocrinol. Metab.* 89: 3922-3931.
- Tunbridge, E.M., et al. 2004. Catechol-o-methyltransferase inhibition improves set-shifting performance and elevates stimulated dopamine release in the rat prefrontal cortex. *J. Neurosci.* 24: 5331-5335.
- Zhu, B.T. 2004. CNS dopamine oxidation and catechol-O-methyltransferase: importance in the etiology, pharmacotherapy, and dietary prevention of Parkinson's disease. *Int. J. Mol. Med.* 13: 343-353.

CHROMOSOMAL LOCATION

Genetic locus: COMT (human) mapping to 22q11.21.

PRODUCT

COMT 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 COMT siRNA (h): sc-43693 and COMT shRNA Plasmid (h): sc-43693-SH as alternate gene silencing products.

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.

APPLICATIONS

COMT shRNA (h) Lentiviral Particles is recommended for the inhibition of COMT 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

COMT (G-4): sc-137253 is recommended as a control antibody for monitoring of COMT 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 COMT gene expression knockdown using RT-PCR Primer: COMT (h)-PR: sc-43693-PR (20 μ l). 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.