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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) 

TGF β 1/2/3 shRNA (h) Lentiviral Particles: sc-44146-V

BACKGROUND

Transforming growth factor β s (TGF β s) were originally discovered due to their ability to promote anchorage-independent growth of rat NRK fibroblasts in the presence of TGF α . It is now realized that TGF β s mediate many cell-cell interactions that occur during embryonic development. Three TGF β s have been identified in mammals. TGF β 1, TGF β 2 and TGF β 3 are each synthesized as precursor proteins that are very similar in that each is cleaved to yield a 112 amino acid polypeptide that remains associated with the latent portion of the molecules. Biologically active TGF β requires dimerization of the monomers (usually homodimers) and release of the latent peptide portion. Overall, the mature region of the TGF β 3 protein has approximately 80% identity to the mature region of both TGF β 1 and TGF β 2. However, the NH₂ terminals or precursor regions of their molecules share only 27% sequence identity.

REFERENCES

1. Todaro, G.J., et al. 1980. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc. Natl. Acad. Sci. USA* 77: 5258-5262.
2. Anzano, M.A., et al. 1983. Sarcoma growth factor from conditioned medium of virally transformed cells is composed of both type α and type β transforming growth factors. *Proc. Natl. Acad. Sci. USA* 80: 6264-6268.
3. Derynck, R., et al. 1985. Human transforming growth factor β cDNA sequence and expression in tumor cell lines. *Nature* 316: 701-705.
4. deMartin, R., et al. 1987. Complementary DNA for human glioblastoma-derived factor β family. *EMBO J.* 6: 3673-3677.
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PRODUCT

TGF β 1/2/3 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 TGF β 1/2/3 siRNA (h): sc-44146 and TGF β 1/2/3 shRNA Plasmid (h): sc-44146-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

TGF β 1/2/3 shRNA (h) Lentiviral Particles is recommended for the inhibition of TGF β 1, TGF β 2 and TGF β 3 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

TGF β 1/2/3 (B-11): sc-166861 is recommended as a control antibody for monitoring of TGF β 1, TGF β 2 and TGF β 3 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TGF β 1, TGF β 2 and TGF β 3 gene expression knockdown using RT-PCR Primer: TGF β 1/2/3 (h)-PR: sc-44146-PR (20 μ l). 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.

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.