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IDO shRNA (h) Lentiviral Particles: sc-45939-V

BACKGROUND

Indoleamine 2,3-dioxygenase (IDO) is an IFN- γ inducible enzyme that catalyzes the degradation of the essential amino acid L-tryptophan to N-formylkynurenine. The gene encoding human IDO maps to chromosome 8p11.21. IDO, also known as INDO, is an important modulator of immunological responses and protects allogeneic concepti from alloreactive maternal lymphocytes. IDO mediates an interesting inhibitory effect of HeLa cells co-cultured with human PBLs. The ILN-2-induced proliferation response of PBLs is diminished in the presence of HeLa cells while an IDO inhibitor negates this effect. Flow cytometric analysis indicates both mature and immature CD123-positive dendritic cells suppress T cell activity using IDO. IDO-transfected cells co-cultured with T cells reduces T cell proliferation. Additionally, adopted transfer of donor T cells reduces donor T cell numbers in IDO-transgenic mice. The pharmacological or genetic manipulation of IDO may be useful for suppressing undesirable T cell response.

REFERENCES

1. Dai, W. and Gupta, S.L. 1990. Molecular cloning, sequencing and expression of human interferon- γ -inducible indoleamine 2,3-dioxygenase cDNA. *Biochem. Biophys. Res. Commun.* 168: 1-8.
2. Najfeld, V., Menninger, J., Muhleman, D., Comings, D.E. and Gupta, S.L. 1993. Localization of indoleamine 2,3-dioxygenase gene (INDO) to chromosome 8p12 \rightarrow p11 by fluorescent *in situ* hybridization. *Cytogenet. Cell Genet.* 64: 231-232.
3. Munn, D.H., Zhou, M., Attwood, J.T., Bondarev, I., Conway, S.J., Marshall, B., Brown, C. and Mellor, A.L. 1998. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281: 1191-1193.

CHROMOSOMAL LOCATION

Genetic locus: IDO1 (human) mapping to 8p11.21.

PRODUCT

IDO 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 IDO siRNA (h): sc-45939 and IDO shRNA Plasmid (h): sc-45939-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

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

IDO (E-11): sc-365517 is recommended as a control antibody for monitoring of IDO 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 IDO gene expression knockdown using RT-PCR Primer: IDO (h)-PR: sc-45939-PR (20 μ l, 515 bp). 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.

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 or our catalog for detailed protocols and support products.