

IDO siRNA (h): sc-45939

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., et al. 1993. Localization of indoleamine 2,3-dioxygenase gene (INDO) to chromosome 8p12→p11 by fluorescent *in situ* hybridization. *Cytogenet. Cell Genet.* 64: 231-232.
3. Munn, D.H., et al. 1998. Prevention of allogeneic fetal rejection by tryptophan catabolism. *Science* 281: 1191-1193.
4. Mellor, A.L., et al. 2002. Cells expressing indoleamine 2,3-dioxygenase inhibit T cell responses. *J. Immunol.* 168: 3771-3776.

CHROMOSOMAL LOCATION

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

PRODUCT

IDO siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IDO shRNA Plasmid (h): sc-45939-SH and IDO shRNA (h) Lentiviral Particles: sc-45939-V as alternate gene silencing products.

For independent verification of IDO (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45939A, sc-45939B and sc-45939C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

IDO siRNA (h) is recommended for the inhibition of IDO expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

IDO (mIDO-48): sc-53978 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).

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° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Li, H., et al. 2014. Human osteoclasts are inducible immunosuppressive cells in response to T cell-derived IFN- γ and CD40 ligand *in vitro*. *J. Bone Miner. Res.* 29: 2666-2675.
2. Rabbani, M.A., et al. 2016. Identification of interferon-stimulated gene proteins that inhibit human parainfluenza virus type 3. *J. Virol.* 90: 11145-11156.
3. Kim, N.S., et al. 2019. LPS enhances CTB-INSULIN induction of IDO1 and IL-10 synthesis in human dendritic cells. *Cell. Immunol.* 338: 32-42.
4. Sharma, M.D., et al. 2021. Inhibition of the BTK-IDO-mTOR axis promotes differentiation of monocyte-lineage dendritic cells and enhances anti-tumor T cell immunity. *Immunity* 54: 2354-2371.e8.
5. Jung, M.Y., et al. 2022. Superinduction of immunosuppressive glioblastoma extracellular vesicles by IFN- γ through PD-L1 and IDO1. *Neurooncol. Adv.* 4: vda017.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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