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CD73 siRNA (h): sc-42862

BACKGROUND

CD73 (also designated ecto-5'-nucleotidase, E5NT, NT, NT5, NTE, eN and eNT) is a glycosyl-phosphatidylinositol (GPI)-anchored adhesion protein that catalyzes the dephosphorylation of extracellular purine and pyrimidine nucleotides to their corresponding bioactive nucleosides. CD73 is a dimer of two identical subunits that depends on GPI to link with the external face of the plasma membrane. Similar to other GPI-anchored proteins, CD73 mediates costimulatory signals in T cell activation. CD73 has few structural variants, yet elicits diverse biological function through differential regulation in endothelial cells (EC), subpopulations of B and T cells, germinal center follicular dendritic cells and on thymic medullary reticular fibroblasts. For example, IgG-mediated neutralization of CD73 interferes with lymphocyte adhesion to EC, and blocks aggregation of germinal center B cells and follicular dendritic cells. Furthermore, IgG-mediated targeting of lymphocyte CD73, but not of endothelial cell CD73, causes shedding of CD73 and tyrosine phosphorylation of proteins.

REFERENCES

1. Yamashita, Y., et al. 1998. CD73 expression and Fyn-dependent signaling on murine lymphocytes. *Eur. J. Immunol.* 28: 2981-2990.
2. Kalsi, K., et al. 2002. Regulation of ecto-5'-nucleotidase by TNF α in human endothelial cells. *Mol. Cell. Biochem.* 232: 113-119.
3. Henttinen, T., et al. 2003. Adherent leukocytes prevent adenosine formation and impair endothelial barrier function by ecto-5'-nucleotidase/CD73-dependent mechanism. *J. Biol. Chem.* 278: 24888-24895.
4. Niemela, J., et al. 2004. IFN- α induced adenosine production on the endo-thelium: a mechanism mediated by CD73 (ecto-5'-nucleotidase) upregulation. *J. Immunol.* 172: 1646-1653.

CHROMOSOMAL LOCATION

Genetic locus: NT5E (human) mapping to 6q14.3.

PRODUCT

CD73 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 CD73 shRNA Plasmid (h): sc-42862-SH and CD73 shRNA (h) Lentiviral Particles: sc-42862-V as alternate gene silencing products.

For independent verification of CD73 (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-42862A, sc-42862B and sc-42862C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

CD73 siRNA (h) is recommended for the inhibition of CD73 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

CD73 (D-12): sc-398260 is recommended as a control antibody for monitoring of CD73 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CD73 gene expression knockdown using RT-PCR Primer: CD73 (h)-PR: sc-42862-PR (20 μ l, 511 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Torres, A., et al. 2019. Extracellular adenosine promotes cell migration/ invasion of glioblastoma stem-like cells through A₃ adenosine receptor activation under hypoxia. *Cancer Lett.* 446: 112-122.

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.