

MECL-1 siRNA (h): sc-42906

BACKGROUND

The 20S proteasome is a protease complex that is responsible for cytosolic protein degradation and generation of peptide ligands for major histocompatibility complex (MHC) class I molecules, either in their final form or in the form of amino-terminally extended precursors. Upon IFN- γ stimulation of cells, three constitutively expressed subunits of the 20S proteasome are replaced by inducible subunits LMP2 (low-molecular mass polypeptide 2), LMP7, and MECL-1 (multicatalytic endopeptidase complex-like-1, LMP10). LMP2, LMP7, and MECL-1 subunits form immunoproteasomes, which are associated with more efficient class I antigen processing and presentation. Independent assortment of LMP-2, LMP-7, and MECL-1 into different proteasome complexes can lead to 36 unique proteasome subsets, which may mediate differences in the cleavage specificities/cleavage motifs of proteins subject to constitutive- and immuno-proteasomes.

REFERENCES

1. Nandi, D., et al. 1996. Identification of MECL-1 (LMP-10) as the third IFN- γ -inducible proteasome subunit. *J. Immunol.* 156: 2361-2364.
2. Frisan, T., et al. 1998. Phenotype-dependent differences in proteasome subunit composition and cleavage specificity in B cell lines. *J. Immunol.* 160: 3281-3289.
3. Schwarz, K., et al. 2000. Overexpression of the proteasome subunits LMP2, LMP7, and MECL-1, but not PA28 α/β , enhances the presentation of an immunodominant lymphocytic choriomeningitis virus T cell epitope. *J. Immunol.* 165: 768-778.
4. Sijts, A.J., et al. 2000. Efficient generation of a hepatitis B virus cytotoxic T lymphocyte epitope requires the structural features of immunoproteasomes. *J. Exp. Med.* 191: 503-514.
5. Toes, R.E., et al. 2001. Discrete cleavage motifs of constitutive and immuno-proteasomes revealed by quantitative analysis of cleavage products. *J. Exp. Med.* 194: 1-12.

CHROMOSOMAL LOCATION

Genetic locus: PSMB10 (human) mapping to 16q22.1.

PRODUCT

MECL-1 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 MECL-1 shRNA Plasmid (h): sc-42906-SH and MECL-1 shRNA (h) Lentiviral Particles: sc-42906-V as alternate gene silencing products.

For independent verification of MECL-1 (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-42906A, sc-42906B and sc-42906C.

PROTOCOLS

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

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

MECL-1 siRNA (h) is recommended for the inhibition of MECL-1 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

MECL-1 (C-2): sc-133236 is recommended as a control antibody for monitoring of MECL-1 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 MECL-1 gene expression knockdown using RT-PCR Primer: MECL-1 (h)-PR: sc-42906-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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