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ERAP1 siRNA (h): sc-43577

BACKGROUND

The endoplasmic reticulum (ER) aminopeptidase 1 (ERAP1) is localized to the lumen of the ER, which removes NH₂-terminal residues from many antigenic precursors for MHC class I peptide presentation. ERAP1 is also designated adipocyte-derived leucine aminopeptidase (A-LAP), puromycin-insensitive leucine-specific aminopeptidase (PILS-AP) and aminopeptidase regulator of TNFR1 shedding (ARTS-1). Peptides presented by MHC class I on the surface of a cell must be eight to eleven residues long and ERAP1 specifically trims peptides of nine amino acids or more. ERAP1 is induced by interferon- γ and encoded for by the ARTS1 gene, which maps to human chromosome 5q15. ERAP1 is thought to inactivate several bioactive peptides, including angiotensin II, and subsequently, may be involved in the regulation of blood pressure. It may have a role in angiogenesis by regulating the proliferation and migration of endothelial cells, and is characterized as a TNFR1 binding protein that promotes TNFR1 shedding.

REFERENCES

1. Saric, T., et al. 2002. An IFN- γ -induced aminopeptidase in the ER, ERAP1, trims precursors to MHC class I-presented peptides. *Nat. Immunol.* 3: 1169-1176.
2. York, I.A., et al. 2002. The ER aminopeptidase ERAP1 enhances or limits antigen presentation by trimming epitopes to 8-9 residues. *Nat. Immunol.* 3: 1177-1184.
3. Hattori, A., et al. 2000. Characterization of recombinant human adipocyte-derived leucine aminopeptidase expressed in Chinese hamster ovary cells. *J. Biochem.* 128: 755-762.
4. Hattori, A., et al. 2001. Genomic organization of the human adipocyte-derived leucine aminopeptidase gene and its relationship to the placental leucine aminopeptidase/oxytocinase gene. *J. Biochem.* 130: 235-241.
5. Cui, X., et al. 2002. Identification of ARTS-1 as a novel TNFR1-binding protein that promotes TNFR1 ectodomain shedding. *J. Clin. Invest.* 110: 515-526.
6. Yamamoto, N., et al. 2002. Identification of 33 polymorphisms in the adipocyte-derived leucine aminopeptidase (ALAP) gene and possible association with hypertension. *Hum. Mutat.* 19: 251-257.

CHROMOSOMAL LOCATION

Genetic locus: ERAP1 (human) mapping to 5q15.

PRODUCT

ERAP1 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 ERAP1 shRNA Plasmid (h): sc-43577-SH and ERAP1 shRNA (h) Lentiviral Particles: sc-43577-V as alternate gene silencing products.

For independent verification of ERAP1 (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-43577A, sc-43577B and sc-43577C.

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

ERAP1 siRNA (h) is recommended for the inhibition of ERAP1 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

ERAP1 (B-10): sc-271823 is recommended as a control antibody for monitoring of ERAP1 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 ERAP1 gene expression knockdown using RT-PCR Primer: ERAP1 (h)-PR: sc-43577-PR (20 μ l, 582 bp). 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.

PROTOCOLS

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