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ERAP1 shRNA (h) Lentiviral Particles: sc-43577-V

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.

CHROMOSOMAL LOCATION

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

PRODUCT

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

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° 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.

APPLICATIONS

ERAP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of ERAP1 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

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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.

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.

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.