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HVEM shRNA (m) Lentiviral Particles: sc-44372-V

BACKGROUND

HVEM (herpes virus entry mediator A), also known as TR2, ATAR, HVEA, LIGHTR or TNFRSF14 (tumor necrosis factor receptor superfamily, member 14), is a 283 amino acid single-pass type I membrane protein that is widely expressed, with highest expression in lung, spleen and thymus. A member of the TNF receptor superfamily, HVEM mediates the entry of herpes simplex virus (HSV) 1 and 2 into T lymphocytes by serving as an attachment site for the HSV envelope glycoprotein D (gD). HVEM acts as a receptor for two cellular ligands, secreted lymphotoxin and LIGHT. A member of the TNF superfamily produced by activated T-cell, LIGHT is suggested to induce apoptosis and suppress tumor formation. Consisting of three TNFR-Cys repeats, HVEM plays a critical role in HSV pathogenesis. HVEM is encoded by a gene located on human chromosome 1, which spans 260 million base pairs, contains over 3,000 genes and comprises nearly 8% of the human genome.

REFERENCES

1. Montgomery, R.I., et al. 1996. Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family. *Cell* 87: 427-436.
2. Marsters, S.A., et al. 1997. Herpesvirus entry mediator, a member of the tumor necrosis factor receptor (TNFR) family, interacts with members of the TNFR-associated factor family and activates the transcription factors NFκB and AP-1. *J. Biol. Chem.* 30: 14029-14032.
3. Whitbeck, J.C., et al. 1997. Glycoprotein D of herpes simplex virus (HSV) binds directly to HVEM, a member of the tumor necrosis factor receptor superfamily and a mediator of HSV entry. *J. Virol.* 71: 6083-6093.
4. Mauri, D.N., et al. 1998. LIGHT, a new member of the TNF superfamily, and lymphotoxin-α are ligands for herpesvirus entry mediator. *Immunity* 8: 21-30.
5. Zhai, Y., et al. 1998. LIGHT, a novel ligand for lymphotoxin-β receptor and TR2/HVEM, induces apoptosis and suppresses *in vivo* tumor formation via gene transfer. *J. Clin. Invest.* 15: 1142-1151.

CHROMOSOMAL LOCATION

Genetic locus: *Tnfrsf14* (mouse) mapping to 4 E2.

PRODUCT

HVEM shRNA (m) 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 μl frozen stock containing 1.0 x 10⁶ 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 HVEM siRNA (m): sc-44372 and HVEM shRNA Plasmid (m): sc-44372-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.

APPLICATIONS

HVEM shRNA (m) Lentiviral Particles is recommended for the inhibition of HVEM expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ 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

HVEM (D-5): sc-365971 is recommended as a control antibody for monitoring of HVEM 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 HVEM gene expression knockdown using RT-PCR Primer: HVEM (m)-PR: sc-44372-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIO SAFETY

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.

PROTOCOLS

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