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SANTA CRUZ BIOTECHNOLOGY, INC.

TEM8 siRNA (h): sc-44144

BACKGROUND

The tripartite toxin secreted by *Bacillus anthraci* is the causative agent of anthrax evading the immune system and killing the host during a systemic infection. Two components of the toxin, odemema factor (OF) and lethal factor (LF) enzymatically modify substrates within the cytosol of mammalian cells. The third component, protective antigen (PA), binds to a cellular receptor, designated ATR (anthrax toxin receptor), which mediates the delivery of the enzymatic components to the cytosol. TEM8 (tumor endothelial marker 8) is one of the tumor specific endothelial markers (TEMs) whose N-terminus encodes ATR. TEM8 is highly expressed in tumor endothelial cells but not in normal endothelial cells. TEMs have elevated expression during tumor angiogenesis. Four TEM genes, TEM1, TEM5, TEM7 and TEM8, encode the TEM proteins, which contain putative transmembrane domains. ATR is a type I membrane protein with an extracellular von Willebrand factor A domain that binds directly to PA. The first 364 amino acids of ATR protein are identical to those of TEM8. However, the C-terminal ends of the ATR and TEM8 proteins are different, presumably due to alternative splicing. A soluble version of von Willebrand factor A domain seems to protect cells from the toxin action.

REFERENCES

1. Leppla, S.H. 1982. Anthrax toxin edema factor: a bacterial adenylate cyclase that increases cAMP concentration in eukaryotic cells. Proc. Natl. Acad. Sci. USA 79: 3162-3166.
2. O'Brien, J., et al. 1985. Effects of anthrax toxin components on human neutrophils. Infect. Immun. 47: 306-310.
3. Duesbery, N.S., et al. 1998. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. Science 280: 734-737.
4. Pellizzari, R., et al. 1999. Anthrax lethal factor cleaves MKK3 in macrophages and inhibits the LPS/IFN-γ-induced release of NO and TNFα. FEBS Lett. 462: 199-204.
5. St Croix, B., et al. 2000. Genes expressed in human tumor endothelium. Science 289: 1197-1202.
6. Bradley, K.A., et al. 2001. Identification of the cellular receptor for anthrax toxin. Nature 414: 225-229.

CHROMOSOMAL LOCATION

Genetic locus: ANTXR1 (human) mapping to 2p13.3.

PRODUCT

TEM8 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TEM8 shRNA Plasmid (h): sc-44144-SH and TEM8 shRNA (h) Lentiviral Particles: sc-44144-V as alternate gene silencing products.

For independent verification of TEM8 (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-44144A, sc-44144B and sc-44144C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TEM8 siRNA (h) is recommended for the inhibition of TEM8 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 µM in 66 µl. 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

TEM8 (4H261): sc-73136 is recommended as a control antibody for monitoring of TEM8 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TEM8 gene expression knockdown using RT-PCR Primer: TEM8 (h)-PR: sc-44144-PR (20 µl, 536 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.