

Themis siRNA (h): sc-95448

BACKGROUND

Themis (thymocyte-expressed molecule involved in selection) is a 641 amino acid nuclear and cytoplasmic protein that exists as three alternatively spliced isoforms. Themis belongs to the themis family and interacts with PLC γ 1, Emt and GRB2. Acting through T-cell antigen receptor (TCR) signaling and through the regulation of calcium influx and phosphorylation of ERK 1, Themis plays a central role in late thymocyte development by controlling both positive and negative T-cell selection. Themis is phosphorylated on Tyr residues quickly after TCR stimulation. The gene that encodes Themis maps to human chromosome 6q22.33. With 170 million base pairs, chromosome 6 comprises nearly 6% of the human genome. Deletion of a portion of the q arm of chromosome 6 is associated with early onset intestinal cancer, suggesting the presence of a cancer susceptibility locus. Additionally, Porphyria cutanea tarda, Parkinson's disease, Stickler syndrome and a susceptibility to bipolar disorder are all associated with genes that map to chromosome 6.

REFERENCES

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- Bläker, H., et al. 2008. Recurrent deletions at 6q in early age of onset non-HNPCC- and non-FAP-associated intestinal carcinomas. Evidence for a novel cancer susceptibility locus at 6q14-q22. *Genes Chromosomes Cancer* 47: 159-164.
- Johnson, A.L., et al. 2009. Themis is a member of a new metazoan gene family and is required for the completion of thymocyte positive selection. *Nat. Immunol.* 10: 831-839.
- Lesourne, R., et al. 2009. Themis, a T cell-specific protein important for late thymocyte development. *Nat. Immunol.* 10: 840-847.
- Fu, G., et al. 2009. Themis controls thymocyte selection through regulation of T cell antigen receptor-mediated signaling. *Nat. Immunol.* 10: 848-856.
- Mayya, V., et al. 2009. Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions. *Sci. Signal.* 2: ra46.

CHROMOSOMAL LOCATION

Genetic locus: THEMIS (human) mapping to 6q22.33.

PRODUCT

Themis 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 Themis shRNA Plasmid (h): sc-95448-SH and Themis shRNA (h) Lentiviral Particles: sc-95448-V as alternate gene silencing products.

For independent verification of Themis (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-95448A, sc-95448B and sc-95448C.

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

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

Themis (A-11): sc-514012 is recommended as a control antibody for monitoring of Themis 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 Themis gene expression knockdown using RT-PCR Primer: Themis (h)-PR: sc-95448-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.

PROTOCOLS

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