SANTA CRUZ BIOTECHNOLOGY, INC.

TPO siRNA (h): sc-39807

BACKGROUND

Thrombopoietin (TPO or THPO), also known as c-Mpl ligand (c-Mpl L), is a cytokine that plays a central role in megakaryopoiesis by influencing the development and maturation of megakaryocytes and platelet production. TPO is expressed by both mature and progenitor megakaryocytes, as well as by human platelets. Human TPO cDNA encodes a 332 amino acid precursor with a 21 amino acid signal peptide which is cleaved to generate the mature protein. TPO isolated from serum ranges in molecular weight, which suggests that the protein is highly glycosylated. TPO exerts its biological effects through the TPO receptor, c-Mpl. Stimulation of c-Mpl with TPO results in the activation of the Janus tyrosine kinase family members, Tyk 2 and JAK2 which in turn phosphorylate Stat5 and Stat3, causing their nuclear translocation and the transcription of Stat responsive genes. The gene encoding TPO maps to human chromosome 3q27.1.

REFERENCES

1. Foster, D.C., et al. 1994. Human thrombopoietin: gene structure, cDNA sequence, expression, and chromosomal localization. Proc. Nat. Acad. Sci. USA 91: 13023-13027.
2. Dorsch, M., et al. 1995. TPO and IL-3 induce overlapping but distinct protein tyrosine phosphorylation in a myeloid precursor cell line. Biochem. Biophys. Res. Commun. 214: 424-431.
3. Kaushansky, K. 1995. Thrombopoietin: the primary regulator of platelet production. Blood 86: 419-431.
4. Chen, J., et al. 1995. Regulation of platelet activation *in vitro* by the c-Mpl ligand, thrombopoietin. Blood 86: 4054-4062.
5. Sasaki, K., et al. 1995. TPO/c-mpl ligand induces tyrosine phosphorylation of multiple cellular proteins including proto-oncogene products, Vav and c-Cbl, and Ras signaling molecules. Biochem. Biophys. Res. Commun. 216: 338-347.
6. Bacon, C.M., et al. 1995. Thrombopoietin (TPO) induces tyrosine phosphorylation and activation of Stat5 and Stat3. FEBS Lett. 370: 63-68.
7. Ezumi, Y., et al. 1995. Thrombopoietin, c-Mpl ligand, induces tyrosine phosphorylation of Tyk2, JAK2, and Stat3, and enhances agonists-induced aggregation in platelets *in vitro*. FEBS Lett. 374: 48-52.

CHROMOSOMAL LOCATION

Genetic locus: THPO (human) mapping to 3q27.1.

PRODUCT

TPO 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 TPO shRNA Plasmid (h): sc-39807-SH and TPO shRNA (h) Lentiviral Particles: sc-39807-V as alternate gene silencing products.

For independent verification of TPO (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-39807A, sc-39807B and sc-39807C.

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

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

TPO (F-3): sc-374045 is recommended as a control antibody for monitoring of TPO 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 TPO gene expression knockdown using RT-PCR Primer: TPO (h)-PR: sc-39807-PR (20 µl). 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.