SANTA CRUZ BIOTECHNOLOGY, INC.

c-Mpl siRNA (m): sc-29854

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 from hematopoietic stem cells. TPO exerts its biological effects through the TPO receptor, c-Mpl. c-Mpl is a member of the cytokine receptor superfamily. Expression of c-Mpl is restricted to hematopoietic tissues and cells, such as bone marrow, spleen, fetal liver and $CD34^+$ cells. 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. Mutations in c-Mpl have been implicated as the cause of certain human disorders, including congenital amegakaryocytic thrombocytopenia (CAMT) and thrombocytopenia with absent radii (TAR) syndrome.

REFERENCES

1. 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.
2. Chen, J., et al. 1995. Regulation of platelet activation *in vitro* by the c-Mpl ligand, thrombopoietin. Blood 86: 4054-4062.
3. Bacon, C.M., et al. 1995. Thrombopoietin (TPO) induces tyrosine phosphorylation and activation of STAT5 and STAT3. FEBS Lett. 370: 63-68.
4. 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.
5. Ballmaier, M., et al. 1998. Defective c-Mpl signaling in the syndrome of thrombocytopenia with absent radii. Stem Cells 16: 177-184.
6. Luoh, S., et al. 2000. Role of the Distal half of the c-Mpl intracellular domain in control of platelet production by thrombopoietin *in vivo*. Mol. Cell. Biol. 20: 507-515.

CHROMOSOMAL LOCATION

Genetic locus: Mpl (mouse) mapping to 4 D2.1.

PRODUCT

c-Mpl siRNA (m) 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 c-Mpl shRNA Plasmid (m): sc-29854-SH and c-Mpl shRNA (m) Lentiviral Particles: sc-29854-V as alternate gene silencing products.

For independent verification of c-Mpl (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-29854A, sc-29854B and sc-29854C.

PROTOCOLS

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

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

c-Mpl siRNA (m) is recommended for the inhibition of c-Mpl expression in mouse 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

c-Mpl (E-7): sc-377417 is recommended as a control antibody for monitoring of c-Mpl 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 c-Mpl gene expression knockdown using RT-PCR Primer: c-Mpl (m)-PR: sc-29854-PR (20 µl, 598 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.