

## c-Mpl siRNA (h): sc-29853

### 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<sup>+</sup> 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 (human) mapping to 1p34.2.

### PRODUCT

c-Mpl 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see c-Mpl shRNA Plasmid (h): sc-29853-SH and c-Mpl shRNA (h) Lentiviral Particles: sc-29853-V as alternate gene silencing products.

For independent verification of c-Mpl (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-29853A, sc-29853B and sc-29853C.

### PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

c-Mpl siRNA (h) is recommended for the inhibition of c-Mpl 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  $\mu$ M in 66  $\mu$ 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

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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> 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 (h)-PR: sc-29853-PR (20  $\mu$ 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.