

Emt siRNA (h): sc-35300

BACKGROUND

The Tec family of non-receptor tyrosine kinases is composed of six proteins designated Tec, Emt (also known as Itk or Tsk), Btk (previously known as Atk, BPK or Emb), Bmx, Txk (also known as Rlk) and Dsrc28C. All members of the family contain SH3 and SH2 domains and, with the exception of Txk and Dsrc28C, also contain a pleckstrin homology (PH) and a Tec homology (TH) domain in their amino termini. Four alternatively spliced forms of Tec are found to be expressed broadly in cells Emt gene product associates with CD28 and becomes activated subsequent to CD28 ligation. Btk is necessary for proper B cell development, and mutations in the gene encoding Btk have been associated with families suffering from X-linked agammaglobulinemia, also referred to as Bruton's disease. The Bmx protein shares a high degree of homology with Btk and seems to be expressed at highest levels in the heart. Txk expression is T cell-specific, while expression of the *Drosophila* Tec homolog, Dsrc28C, is developmentally regulated.

REFERENCES

1. Yamada, N., et al. 1993. Structure and expression of novel protein tyrosine kinases, Emb and Emt, in hematopoietic cells. *Biochem. Biophys. Res. Commun.* 192: 231-240.
2. Thomas, J.D., et al. 1993. Colocalization of X-linked agammaglobulinemia and X-linked immunodeficiency genes. *Science* 261: 355-358.
3. Tamagnone, L., et al. 1994. Bmx, a novel nonreceptor tyrosine kinase gene of the Btk/Itk/Tec/Txk family located in chromosome Xp22.2. *Oncogene* 9: 3683-3688.
4. Haire, R.N., et al. 1994. Txk, a novel human tyrosine kinase expressed in T cells shares sequence identity with Tec family kinases and maps to 4p12. *Hum. Mol. Genet.* 3: 897-901.
5. August, A., et al. 1994. CD28 is associated with and induces the immediate tyrosine phosphorylation and activation of the Tec family kinase Itk/Emt in the human Jurkat leukemic T-cell line. *Proc. Natl. Acad. Sci. USA* 91: 9347-9351.
6. Hu, Q., et al. 1995. Identification of Rlk, a novel protein tyrosine kinase with predominant expression in the T cell lineage. *J. Biol. Chem.* 270: 1928-1934.

CHROMOSOMAL LOCATION

Genetic locus: ITK (human) mapping to 5q33.3.

PRODUCT

Emt 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 Emt shRNA Plasmid (h): sc-35300-SH and Emt shRNA (h) Lentiviral Particles: sc-35300-V as alternate gene silencing products.

For independent verification of Emt (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-35300A, sc-35300B and sc-35300C.

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

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

Emt (2F12): sc-23902 is recommended as a control antibody for monitoring of Emt 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 MarkerTM 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 Emt gene expression knockdown using RT-PCR Primer: Emt (h)-PR: sc-35300-PR (20 μ l, 462 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.