

TGase1 siRNA (m): sc-37513

BACKGROUND

Terminally differentiating mammalian epidermal cells acquire an insoluble, 10 to 20 nm thick protein deposit on the intracellular surface of the plasma membrane known as the cross-linked cell envelope (CE). The CE is a component of the epidermis that is generated through formation of disulfide bonds and γ -glutamyl-lysine isodipeptide bonds, which are formed by the action of transglutaminases (TGases). TGases are intercellularly localizing, Ca^{2+} -dependent enzymes that catalyze the formation of isopeptide bonds by transferring an amine on to glutamyl residues, thereby cross-linking glutamine residues and lysine residues in substrate proteins. TGases influence numerous biological processes, including blood coagulation, epidermal differentiation, seminal fluid coagulation, fertilization, cell differentiation and apoptosis. Human keratinocyte transglutaminase (TGase1) is a membrane associated, 817 amino acid protein. Human tissue transglutaminase (TGase2) is an endothelial cell specific, 687 amino acid protein.

REFERENCES

1. Gentile, V., et al. 1991. Isolation and characterization of cDNA clones to mouse macrophage and human endothelial cell tissue transglutaminases. *J. Biol. Chem.* 266: 478-483.
2. Yamanishi, K., et al. 1991. Molecular cloning of human epidermal transglutaminase cDNA from keratinocytes in culture. *Biochem. Biophys. Res. Commun.* 175: 906-913.
3. Kim, I.G., et al. 1992. Structure and organization of the human transglutaminase 1 gene. *J. Biol. Chem.* 267: 7710-7717.
4. Ueki, S., et al. 1996. Dual functions of transglutaminase in novel cell adhesion. *J. Cell Sci.* 109: 2727-2735.
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CHROMOSOMAL LOCATION

Genetic locus: Tgm1 (mouse) mapping to 14 C3.

PRODUCT

TGase1 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 TGase1 shRNA Plasmid (m): sc-37513-SH and TGase1 shRNA (m) Lentiviral Particles: sc-37513-V as alternate gene silencing products.

For independent verification of TGase1 (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-37513A, sc-37513B and sc-37513C.

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

TGase1 siRNA (m) is recommended for the inhibition of TGase1 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

TGase1 (A-5): sc-365821 is recommended as a control antibody for monitoring of TGase1 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 TGase1 gene expression knockdown using RT-PCR Primer: TGase1 (m)-PR: sc-37513-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.