SANTA CRUZ BIOTECHNOLOGY, INC.

TNAP siRNA (m): sc-38922

BACKGROUND

Alkaline phosphatases (AP) are glycosyl-phosphatidylinositol (GPI)-anchored, dimeric, Zn^{2+} metallated glycoproteins that catalyze the hydrolysis of phospho-monoesters into an inorganic phosphate and an alcohol. There are at least four distinct but related alkaline phosphatases: intestinal (IAP), placental (PLAP), placental-like (ALP-1 or GCAP), and tissue non-specific (TNAP). The first three are located together on chromosome 2 while the tissue non-specific form is located on chromosome 1. TNAP is widely expressed in liver, kidney, bone, stomach and colon, and is, therefore referred to as the tissue non-specific form of AP. TNAP, in conjuntion with plasma cell membrane glycoprotein-1, function in bone mineralization; however, mice that lack a functional form of TNAP show normal skeletal development. This enzyme has been linked directly to a disorder known as hypophosphatasia, a rare inborn disorder that is characterized by defective bone mineraliation and includes skeletal defects. Human gene encoding TNAP maps to chromosome 1p36.12.

REFERENCES

1. Shao, J.S., et al. 2000. Effect of tissue non-specific alkaline phosphatase in maintenance of structure of murine colon and stomach. Microsc. Res. Tech. 51: 121-128.
2. Johnson, K.A., et al. 2000. Osteoblast tissue-nonspecific alkaline phosphatase antagonizes and regulates PC-1. Am. J. Physiol. Regul. Integr. Comp. Physiol. 279: R1365-R1377.
3. Mornet, E., et al. 2001. Structural evidence for a functional role of human tissue nonspecific alkaline phosphatase in bone mineralization. J. Biol. Chem. 276: 31171-31178.
4. Harada, T., et al. 2002. Heat shock induces intestinal-type alkaline phosphatase in rat IEC-18 cells. Am. J. Physiol. Gastrointest. Liver Physiol. 284: G255-G262.
5. Hessle, L., et al. 2002. Tissue-nonspecific alkaline phosphatase and plasma cell membrane glycoprotein-1 are central antagonistic regulators of bone mineralization. Proc. Natl. Acad. Sci. USA 99: 9445-9449.

CHROMOSOMAL LOCATION

Genetic locus: Alpl (mouse) mapping to 4 D3.

PRODUCT

TNAP 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 TNAP shRNA Plasmid (m): sc-38922-SH and TNAP shRNA (m) Lentiviral Particles: sc-38922-V as alternate gene silencing products.

For independent verification of TNAP (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-38922A, sc-38922B and sc-38922C.

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

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

TNAP (F-4): sc-166261 is recommended as a control antibody for monitoring of TNAP 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 TNAP gene expression knockdown using RT-PCR Primer: TNAP (m)-PR: sc-38922-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.