SANTA CRUZ BIOTECHNOLOGY, INC.

HOTTL siRNA (h): sc-62474

BACKGROUND

HOTTL (Tubulin-tyrosine ligase-like protein 3) is a 352 amino acid protein encoded by the human gene TTLL3. HOTTL is believed to be part of the Tubulin-tyrosine ligase family and contains one TTL domain. The TTL domain is an approximately 350 amino acid module present in a family of eukaryotic proteins that could catalyze ligations of diverse amino acids to tubulins or other substrates. The TTL domain contains ATP-grasp-like motifs that correspond to the ATP/Mg^{2+} binding site typical of enzymes with ATP-dependent carboxylate-amine/thiol ligase activity. Subject to several post-translational modifications, assembled microtubules are detyrosinated over time at the C-terminus of α-tubulin. After microtubular disassembly TTL proteins restore tyrosine residues back to the detyrosinated tubulin leading to a cycle of detyrosination/tyrosination. HOTTL also may play a role in tumor cell regulation.

REFERENCES

1. Galperin, M.Y., et al. 1998. A diverse superfamily of enzymes with ATP-dependent carboxylate-amine/thiol ligase activity. Protein Sci. 6: 2639-2643.
2. Regnard, C., et al. 1998. Tubulin polyglutamylase: partial purification and enzymatic properties. Biochemistry 37: 8395-8404.
3. Janke, C., et al. 2005. Tubulin polyglutamylase enzymes are members of the TTL domain protein family. Science 308: 1758-1762.
4. Sjöblom, T., et al. 2006. The consensus coding sequences of human breast and colorectal cancers. Science 314: 268-274.
5. Sweet, T.J., et al. 2007. Microtubule disruption stimulates P-body formation. RNA 13: 493-502.
6. van Dijk, J., et al. 2007. A targeted multienzyme mechanism for selective microtubule polyglutamylation. Mol. Cell 26: 437-448.

CHROMOSOMAL LOCATION

Genetic locus: TTLL3 (human) mapping to 3p25.3.

PRODUCT

HOTTL 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HOTTL shRNA Plasmid (h): sc-62474-SH and HOTTL shRNA (h) Lentiviral Particles: sc-62474-V as alternate gene silencing products.

For independent verification of HOTTL (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-62474A, sc-62474B and sc-62474C.

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.

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

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

HOTTL (F-8): sc-166483 is recommended as a control antibody for monitoring of HOTTL 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 HOTTL gene expression knockdown using RT-PCR Primer: HOTTL (h)-PR: sc-62474-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.