SANTA CRUZ BIOTECHNOLOGY, INC.

KLHL20 siRNA (h): sc-88301

BACKGROUND

KLHL20 (kelch-like 20), also known as KHLHX, KLEIP or KLHLX, is a 609 amino acid protein that is related to the *Drosophila* kelch protein, which is required to maintain Actin organization in ovarian RING canals. Mutations affecting kelch function result in failure of kelch to associate with the RING canals, causing subsequent female sterility. Human KLHL20 protein contains six kelch repeats, one BACK (BTB/kelch associated) domain and one BTB (POZ) domain. The BTB (broad-complex, tramtrack and bric a brac) domain, also known as the POZ (poxvirus and zinc finger) domain, is an N-terminal homodimerization domain that contains multiple copies of kelch repeats and/or C_2H_2-type zinc fingers. Proteins that contain BTB domains are thought to be involved in transcriptional regulation via control of chromatin structure and function. KLHL20 is a probable substrate-specific adapter of an E3 ubiquitin-protein ligase complex, which mediates the ubiquitination and subsequent proteasomal degradation of target proteins.

REFERENCES

1. Albagli, O., et al. 1995. The BTB/POZ domain: a new protein-protein interaction motif common to DNA- and Actin-binding proteins. Cell Growth Differ. 6: 1193-1198.
2. Robinson, D.N. and Cooley, L. 1997. *Drosophila* kelch is an oligomeric ring canal actin organizer. J. Cell Biol. 138: 799-810.
3. Melnick, A., et al. 2000. In-depth mutational analysis of the promyelocytic leukemia zinc finger BTB/POZ domain reveals motifs and residues required for biological and transcriptional functions. Mol. Cell. Biol. 20: 6550-6567.
4. Adams, J., et al. 2000. The kelch repeat superfamily of proteins: propellers of cell function. Trends Cell Biol. 10: 17-24.
5. Kelso, R.J., et al. 2002. *Drosophila* kelch regulates actin organization via Src64-dependent tyrosine phosphorylation. J. Cell Biol. 156: 703-713.
6. Prag, S. and Adams, J.C. 2003. Molecular phylogeny of the kelch-repeat superfamily reveals an expansion of BTB/kelch proteins in animals. BMC Bioinformatics 4: 42.
7. Geyer, R., et al. 2003. BTB/POZ domain proteins are putative substrate adaptors for cullin 3 ubiquitin ligases. Mol. Cell 12: 783-790.

CHROMOSOMAL LOCATION

Genetic locus: KLHL20 (human) mapping to 1q25.1.

PRODUCT

KLHL20 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 KLHL20 shRNA Plasmid (h): sc-88301-SH and KLHL20 shRNA (h) Lentiviral Particles: sc-88301-V as alternate gene silencing products.

For independent verification of KLHL20 (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-88301A, sc-88301B and sc-88301C.

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

KLHL20 siRNA (h) is recommended for the inhibition of KLHL20 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

KLHL20 (D-7): sc-515381 is recommended as a control antibody for monitoring of KLHL20 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 KLHL20 gene expression knockdown using RT-PCR Primer: KLHL20 (h)-PR: sc-88301-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.