

KLHL12 siRNA (m): sc-146513

BACKGROUND

KLHL12 (kelch-like protein 12), also known as C3IP1 (CUL3-interacting protein 1), DKIR or FLJ27152, is a 568 amino acid protein that is a component of an ubiquitin-protein E3 ligase complex, which includes at least CUL-3. KLHL12 is a substrate-specific adapter for the complex, which negatively regulates the Wnt signaling pathway via the targeted ubiquitination and subsequent proteolysis of Dvl-3. KLHL12 contains six Kelch repeats and one BTB (POZ) domain, which is required for interaction with CUL-3. KLHL12 has highest expression in testis, with lower levels found in the submandibular salivary gland. The gene that encodes KLHL12 maps to human chromosome 1q32.1.

REFERENCES

1. Mai, A., Jung, S.K. and Yonehara, S. 2004. hDKIR, a human homologue of the *Drosophila* kelch protein, involved in a ring-like structure. *Exp. Cell Res.* 300: 72-83.
2. Lim, J., Hao, T., Shaw, C., Patel, A.J., Szabó, G., Rual, J.F., Fisk, C.J., Li, N., Smolyar, A., Hill, D.E., Barabási, A.L., Vidal, M. and Zoghbi, H.Y. 2006. A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration. *Cell* 125: 801-814.
3. Angers, S., Thorpe, C.J., Biechele, T.L., Goldenberg, S.J., Zheng, N., MacCoss, M.J. and Moon, R.T. 2006. The KLHL12-Cullin-3 ubiquitin ligase negatively regulates the Wnt- β -catenin pathway by targeting Dishevelled for degradation. *Nat. Cell Biol.* 8: 348-357.
4. Rondou, P., Haegeman, G., Vanhoenacker, P. and Van Craenenbroeck, K. 2008. BTB Protein KLHL12 targets the dopamine D4 receptor for ubiquitination by a Cul3-based E3 ligase. *J. Biol. Chem.* 283: 11083-11096.
5. Ehret, G.B., O'Connor, A.A., Weder, A., Cooper, R.S. and Chakravarti, A. 2009. Follow-up of a major linkage peak on chromosome 1 reveals suggestive QTLs associated with essential hypertension: GenNet study. *Eur. J. Hum. Genet.* 17: 1650-1657.

CHROMOSOMAL LOCATION

Genetic locus: Klhl12 (mouse) mapping to 1 E4.

PRODUCT

KLHL12 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see KLHL12 shRNA Plasmid (m): sc-146513-SH and KLHL12 shRNA (m) Lentiviral Particles: sc-146513-V as alternate gene silencing products.

For independent verification of KLHL12 (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-146513A, sc-146513B and sc-146513C.

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 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

KLHL12 siRNA (m) is recommended for the inhibition of KLHL12 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 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

KLHL12 (D-1): sc-514874 is recommended as a control antibody for monitoring of KLHL12 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 KLHL12 gene expression knockdown using RT-PCR Primer: KLHL12 (m)-PR: sc-146513-PR (20 μ l). 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.