

Skeletrophin siRNA (h): sc-63036

BACKGROUND

Skeletrophin, also known as MIB2 (mind bomb homolog 2), SKD, ZZANK1 or ZZZ5, is an E3 ubiquitin-protein ligase that regulates Notch signaling via protein degradation. Expressed in heart, brain, kidney and skeletal muscle, Skeletrophin positively regulates Notch signaling by ubiquitinating the intracellular domain of Delta receptors, which act as ligands for Notch proteins. This ubiquitination facilitates transendocytosis of the Notch extracellular domain which, in turn, cues transportation of the Notch intracellular domain to the nucleus, where it activates a variety of genes. Skeletrophin localizes to the endosome and is downregulated in primary skin melanomas. Treatment of melanoma cells with 5'-aza-2-deoxycytidine, a demethylating agent, increases Skeletrophin expression, suggesting that downregulation is caused by methylation of the Skeletrophin gene. Six isoforms exist due to alternative splicing events.

REFERENCES

1. Takeuchi, T., et al. 2003. Down regulation of a novel Actin-binding molecule, Skeletrophin, in malignant melanoma. *Am. J. Pathol.* 163: 1395-1404.
2. Koo, B.K., et al. 2005. Mind bomb-2 is an E3 ligase for Notch ligand. *J. Biol. Chem.* 280: 22335-22342.
3. Takeuchi, T., et al. 2005. Skeletrophin, a novel RING molecule controlled by the chromatin remodeling complex, is downregulated in malignant melanoma. *DNA Cell Biol.* 24: 339-344.
4. Takeuchi, T., et al. 2005. Skeletrophin, a novel ubiquitin ligase to the intracellular region of Jagged-2, is aberrantly expressed in multiple myeloma. *Am. J. Pathol.* 166: 1817-1826.

CHROMOSOMAL LOCATION

Genetic locus: MIB2 (human) mapping to 1p36.33.

PRODUCT

Skeletrophin 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Skeletrophin shRNA Plasmid (h): sc-63036-SH and Skeletrophin shRNA (h) Lentiviral Particles: sc-63036-V as alternate gene silencing products.

For independent verification of Skeletrophin (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-63036A, sc-63036B and sc-63036C.

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

Skeletrophin siRNA (h) is recommended for the inhibition of Skeletrophin 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 μ M in 60 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

Skeletrophin (D-3): sc-515902 is recommended as a control antibody for monitoring of Skeletrophin 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 Skeletrophin gene expression knockdown using RT-PCR Primer: Skeletrophin (h)-PR: sc-63036-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bai, J., et al. 2018. Ectopic expression of E3 ubiquitin-protein ligase 2 in glioma and enhances resistance to apoptosis through activating nuclear factor κ -light-chain-enhancer of B cells. *Oncol. Lett.* 16: 4391-4399.

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.