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ULK1 shRNA (m) Lentiviral Particles: sc-44849-V

BACKGROUND

ULK1 and ULK2 (for UNC-51-like kinase) encode similar amino-terminal serine/threonine kinase domains, a proline/serine-rich (PS) domain, and a species conserved carboxyl-terminal domain. Both share homology with the UNC-51 kinase from *Caenorhabditis elegans* and the APG1 kinase in yeast, which are involved in axonal extension and growth, and autophagy, respectively. ULK1 maps to human chromosome 12q24.3 and is ubiquitously expressed. ULK2, also widely expressed, maps to mouse chromosome 11B1.3 and is expected to have a similar molecular weight as ULK1 in human. ULK1 and ULK2 are thought to auto-phosphorylate the PS domain *in vitro*, and the significant homology among vertebrates suggest that ULK1 and ULK2 are involved in the regulation of fundamental biological processes.

REFERENCES

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- Matsuura, A., et al. 1997. *apg1p*, a novel protein kinase required for the autophagic process in *Saccharomyces cerevisiae*. *Gene* 192: 245-250.
- Kuroyanagi, H., et al. 1998. Human ULK1, a novel serine/threonine kinase related to UNC-51 kinase of *Caenorhabditis elegans*: cDNA cloning, expression, and chromosomal assignment. *Genomics* 51: 76-85.
- Yan, J., et al. 1998. Identification of mouse ULK1, a novel protein kinase structurally related to *C. elegans* UNC-51. *Biochem. Biophys. Res. Commun.* 246: 222-227.
- Yan, J., et al. 1999. Mouse ULK2, a novel member of the UNC-51-like protein kinases: unique features of functional domains. *Oncogene* 18: 5850-5859.
- Tomoda, T., et al. 1999. A mouse serine/threonine kinase homologous to *C. elegans* UNC51 functions in parallel fiber formation of cerebellar granule neurons. *Neuron* 24: 833-846.

CHROMOSOMAL LOCATION

Genetic locus: Ulk1 (mouse) mapping to 5 F.

PRODUCT

ULK1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ULK1 siRNA (m): sc-44849 and ULK1 shRNA Plasmid (m): sc-44849-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

ULK1 shRNA (m) Lentiviral Particles is recommended for the inhibition of ULK1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ULK1 (H-240): sc-33182 is recommended as a control antibody for monitoring of ULK1 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ULK1 gene expression knockdown using RT-PCR Primer: ULK1 (m)-PR: sc-44849-PR (20 μ l, 570 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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