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MAP LC3 β shRNA (h) Lentiviral Particles: sc-43390-V

BACKGROUND

Microtubule-associated proteins (MAPs) regulate microtubule stability and play critical roles in neuronal development and in maintaining the balance between neuronal plasticity and rigidity. MAP-light chain 3 β (MAP LC3 β) and MAP-light chain 3 α (MAP LC3 α) are subunits of both MAP1A and MAP1B. MAP LC3 β , a homolog of Apg8p, is essential for autophagy and associated to the autophagosome membranes after processing. Two forms of LC3 β , the cytosolic LC3-I and the membrane-bound LC3-II, are produced post-translationally. LC3-I is formed by the removal of the C-terminal 22 amino acids from newly synthesized LC3 β , followed by the conversion of a fraction of LC3-I into LC3-II. LC3 enhances fibronectin mRNA translation in ductus arteriosus cells through association with 60S ribosomes and binding to an AU-rich element in the 3' untranslated region of fibronectin mRNA. This facilitates sorting of fibronectin mRNA onto rough endoplasmic reticulum and translation. MAP LC3 β may also be involved in formation of autophagosomal vacuoles. It is expressed primarily in heart, testis, brain and skeletal muscle.

REFERENCES

1. Fink, J.K., et al. 1996. Human microtubule-associated protein 1A (MAP1A) gene: genomic organization, cDNA sequence, and developmental and tissue-specific expression. *Genomics* 35: 577-585.
2. Mann, S.S., et al. 1996. Gene localization and developmental expression of light chain 3: a common subunit of microtubule-associated protein 1A (MAP1A) and MAP1B. *J. Neurosci. Res.* 43: 535-544.
3. Zhou, B., et al. 1997. Microtubule-associated protein 1 light chain 3 is a Fibronectin mRNA-binding protein linked to mRNA translation in lamb vascular smooth muscle cells. *J. Clin. Invest.* 100: 3070-3082.

CHROMOSOMAL LOCATION

Genetic locus: MAP1LC3B (human) mapping to 16q24.2.

PRODUCT

MAP LC3 β shRNA (h) 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 MAP LC3 β siRNA (h): sc-43390 and MAP LC3 β shRNA Plasmid (h): sc-43390-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.

PROTOCOLS

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

APPLICATIONS

MAP LC3 β shRNA (h) Lentiviral Particles is recommended for the inhibition of MAP LC3 β expression in human 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

MAP LC3 β (N-20): sc-16755 is recommended as a control antibody for monitoring of MAP LC3 β 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MAP LC3 β gene expression knockdown using RT-PCR Primer: MAP LC3 β (h)-PR: sc-43390-PR (20 μ l, 501 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.