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MAFbx siRNA (h): sc-43906

BACKGROUND

Muscle atrophy F-box (MAFbx) is an E3 ubiquitin ligase that initiates ATP-dependent ubiquitin-mediated proteolysis and promotes muscle atrophy. MAFbx transcript is abundant in cardiac and skeletal muscle undergoing atrophy. MAFbx^{-/-} mice are resistant to muscle atrophy. MAFbx is thought to recognize and bind to some phosphorylated proteins and promote their ubiquitination and degradation during skeletal muscle atrophy. It interacts with MyoD by ubiquitination via a sequence found in transcriptional coactivators and therefore may play an important role in the course of muscle differentiation by determining the abundance of MyoD. MAFbx is specifically expressed in cardiac and skeletal muscle.

REFERENCES

1. Bodine, S.C., et al. 2001. Identification of ubiquitin ligases required for skeletal muscle atrophy. *Science* 294: 1704-1708.
2. Dehoux, M.J., et al. 2003. Induction of MafBx and Murf ubiquitin ligase mRNAs in rat skeletal muscle after LPS injection. *FEBS Lett.* 544: 214-217.
3. Glass, D.J. 2003. Signalling pathways that mediate skeletal muscle hypertrophy and atrophy. *Nat. Cell. Biol.* 5: 87-90.
4. Dehoux, M., et al. 2004. Role of the Insulin-like growth factor I decline in the induction of atrogen-1/MAFbx during fasting and diabetes. *Endocrinology* 145:4806-4812.
5. Rourke, B.C., et al. 2004. Myosin isoform expression and MAFbx mRNA levels in hibernating golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiol. Biochem. Zool.* 77: 582-593.
6. Tintignac, L.A., et al. 2005. Degradation of MyoD mediated by the SCF (MAFbx) ubiquitin ligase. *J. Biol. Chem.* 280: 2847-2856.

CHROMOSOMAL LOCATION

Genetic locus: FBX032 (human) mapping to 8q24.13.

PRODUCT

MAFbx 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 MAFbx shRNA Plasmid (h): sc-43906-SH and MAFbx shRNA (h) Lentiviral Particles: sc-43906-V as alternate gene silencing products.

For independent verification of MAFbx (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-43906A, sc-43906B and sc-43906C.

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.

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

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

MAFbx (F-9): sc-166806 is recommended as a control antibody for monitoring of MAFbx 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 MAFbx gene expression knockdown using RT-PCR Primer: MAFbx (h)-PR: sc-43906-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.