SANTA CRUZ BIOTECHNOLOGY, INC.

Cas-L siRNA (m): sc-40795

BACKGROUND

Cas family proteins are adhesion docking molecules that mediate protein-protein interactions and contribute to a number of signal transduction pathways. Cas-L (also designated human enhancer of filamentation (HEF1) and neural precursor cell expressed, developmentally down-regulated 9 (NEDD9), participates in integrin and growth factor signaling pathways that regulate growth, motility and apoptosis. Cas-L consists of two isoforms, p105 and p115. The larger molecular weight form is a result of Ser/Thr phosphorylation. Cas-L phosphorylation is dependent on cell adhesion and Src kinase activity. Cas-L acts as a downstream effector of FAK in the invasive behavior of glioblastoma cells. TGFβ1 regulates Cas-L gene expression and influences phosphorylation. Adhesion-dependent Actin organization regulates proteasomal turnover of Cas-L through the activity of PP2A. Tyrosine phosphorylated Cas-L can bind FAK in dendrite and soma of neurons after ischemia. Cas-L can promote neurite outgrowth of PC-12 cells.

REFERENCES

1. Law, S.F., et al. 2000. The docking protein HEF1 is an apoptotic mediator at focal adhesion sites. Mol. Cell. Biol. 20: 5184-5195.
2. Liu, X., et al. 2000. A novel ability of Smad3 to regulate proteasomal degradation of a Cas family member HEF1. Embo. J. 19: 6759-6769.
3. Zheng, M., et al. 2002. Regulation of HEF1 expression and phosphorylation by TGFβ1 and cell adhesion. J. Biol. Chem. 277: 39599-39608.
4. Natarajan, M., et al. 2005. HEF1 is a necessary and specific downstream effector of FAK that promotes the migration of glioblastoma cells. Oncogene 12: 1721-1732.
5. Iwata, S., et al. 2005. HTLV-I Tax induces and associates with Crk-associated substrate lymphocyte type (Cas-L). Oncogene 24: 1262-1271.
6. Pugacheva, E.N., et al. 2005. The focal adhesion scaffolding protein HEF1 regulates activation of the Aurora-A and Nek2 kinases at the centrosome. Nat. Cell Biol. 7: 937-946.
7. Sasaki, T., et al. 2005. NEDD9 protein, a Cas-L homologue, is up-regulated after transient global ischemia in rats: possible involvement of NEDD9 in the differentiation of neurons after ischemia. Stroke 36: 2457-2462.

CHROMOSOMAL LOCATION

Genetic locus: Nedd9 (mouse) mapping to 13 A3.3.

PRODUCT

Cas-L 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cas-L shRNA Plasmid (m): sc-40795-SH and Cas-L shRNA (m) Lentiviral Particles: sc-40795-V as alternate gene silencing products.

For independent verification of Cas-L (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-40795A, sc-40795B and sc-40795C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Cas-L siRNA (m) is recommended for the inhibition of Cas-L 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 µM in 66 µl. 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

Cas-L (2G9): sc-33659 is recommended as a control antibody for monitoring of Cas-L 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Cas-L gene expression knockdown using RT-PCR Primer: Cas-L (m)-PR: sc-40795-PR (20 µl). 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.

PROTOCOLS

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