SANTA CRUZ BIOTECHNOLOGY, INC.

Ajuba siRNA (m): sc-60067

BACKGROUND

The LIM protein Ajuba (JUB), a member of the Zyxin family, mediates various cellular events. Ajuba is a component of the IL-1 signaling pathway modulating IL-1-induced NFκB activation by influencing the assembly and activity of the αPKC/p62/TRAF6 multiprotein signaling complex. Ajuba also plays a role in cadherin-mediated cell-cell adhesion and influences cell migration by regulating PIP2 synthesis through direct activation of PIPKIα activity. Differentiating mouse embryonic stem cells show elevated Ajuba transcription. In adult mouse tissues, Ajuba is present in skin, brain and genitourinary organs. Immunofluorescence analysis of unsynchronized HeLa cells shows cytoplasmic staining. In cells synchronized at G_2/M, Ajuba localizes to the centrosome, where it complexes with LATS2 to regulate the organization of the spindle apparatus through recruitment of γ Tubulin.

REFERENCES

1. Goyal, R.K., et al. 1999. Ajuba, a novel LIM protein, interacts with GRB2, augments mitogen-activated protein kinase activity in fibroblasts and promotes meiotic maturation of *Xenopus* oocytes in a GRB2- and Ras-dependent manner. Mol. Cell. Biol. 19: 4379-4389.
2. Marie, H., et al. 2002. The amino-terminus of the glial glutamate transporter GLT-1 interacts with the LIM protein Ajuba. Mol. Cell. Neurosci. 19: 152-164.
3. Petit, M.M., et al. 2005. The tumor suppressor Scrib interacts with the Zyxin-related protein LPP, which shuttles between cell adhesion sites and the nucleus. BMC Cell Biol. 6: 1.
4. Benzinger, A., et al 2005. Targeted proteomic analysis of 14-3-3 σ, a p53 effector commonly silenced in cancer. Mol. Cell. Proteomics 4: 785-795.
5. Feng, Y. and Longmore, G.D. 2005. The LIM protein Ajuba influences interleukin-1-induced NFκB activation by affecting the assembly and activity of the PKC ζ/p62/TRAF6 signaling complex. Mol. Cell. Biol. 25: 4010-4022.
6. Pratt, S.J., et al. 2005. The LIM protein Ajuba influences p130Cas localization and Rac1 activity during cell migration. J. Cell Biol. 168: 813-824.

CHROMOSOMAL LOCATION

Genetic locus: Jub (mouse) mapping to 14 C3.

PRODUCT

Ajuba 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 Ajuba shRNA Plasmid (m): sc-60067-SH and Ajuba shRNA (m) Lentiviral Particles: sc-60067-V as alternate gene silencing products.

For independent verification of Ajuba (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-60067A, sc-60067B and sc-60067C.

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

Ajuba siRNA (m) is recommended for the inhibition of Ajuba 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

Ajuba (B-3): sc-398008 is recommended as a control antibody for monitoring of Ajuba 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 Ajuba gene expression knockdown using RT-PCR Primer: Ajuba (m)-PR: sc-60067-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.