SANTA CRUZ BIOTECHNOLOGY, INC.

PJA1 siRNA (h): sc-91297

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The Power to Question

BACKGROUND

Ubiquitinization is an important cellular degradation process requiring sequential reactions that are mediated by three enzymes: E1, E2 and E3. PJA1, also known as Praja1 and RING finger protein 70, is a 643 amino acid E2-dependent E3-ubiquitin ligase that is abundantly expressed in regions of the brain including cerebellum, medulla, cerebral cortex, putamen, occipital pole, temporal lobe and frontal lobe. Through interaction and activation with the E2-ubiquitin ligase UBC4, PJA1 mediates substrate-specific ubiquitization via its RING finger domain. The gene encoding PJA1 may be a candidate gene for X-linked mental retardations (MRXs), such as craniofrontonasal syndrome, due to its location on the X chromosome that is frequently found mutated in MRX patients. Overexpression of PJA1 in gastrointestinal cancers suggests that it may be responsible for the degradation of spectrin β II, a protein that exhibits anti-oncogenic activity. There are two named isoforms of PJA1 that exist as a result of alternative splicing events.

REFERENCES

1. Mishra, L., et al. 1997. Praja1, a novel gene encoding a RING-H2 motif in mouse development. Oncogene 15: 2361-2368.
2. Yu, P., et al. 2002. PJA1, encoding a RING-H2 finger ubiquitin ligase, is a novel human X chromosome gene abundantly expressed in brain. Genomics 79: 869-874.
3. Sasaki, A., et al. 2002. A RING finger protein Praja1 regulates Dlx-5-dependent transcription through its ubiquitin ligase activity for the Dlx/Msx-interacting MAGE/Necdin family protein, Dlxin-1. J. Biol. Chem. 277: 22541-22546.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 300420. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Mishra, L., et al. 2005. The role of PRAJA and ELF in TGF-β signaling and gastric cancer. Cancer Biol. Ther. 4: 694-699.
6. Saha, T., et al. 2006. RING finger-dependent ubiquitination by PRAJA is dependent on TGF-β and potentially defines the functional status of the tumor suppressor ELF. Oncogene 25: 693-705.

CHROMOSOMAL LOCATION

Genetic locus: PJA1 (human) mapping to Xq13.1.

PRODUCT

PJA1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PJA1 shRNA Plasmid (h): sc-91297-SH and PJA1 shRNA (h) Lentiviral Particles: sc-91297-V as alternate gene silencing products.

For independent verification of PJA1 (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-91297A, sc-91297B and sc-91297C.

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

PJA1 siRNA (h) is recommended for the inhibition of PJA1 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 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

PJA1 (3E10): sc-517068 is recommended as a control antibody for monitoring of PJA1 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 PJA1 gene expression knockdown using RT-PCR Primer: PJA1 (h)-PR: sc-91297-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.