SANTA CRUZ BIOTECHNOLOGY, INC.

ValRS siRNA (h): sc-76887

BACKGROUND

The fidelity of protein synthesis requires efficient discrimination of amino acid substrates by aminoacyl-tRNA synthetases. ValRS (valyl-tRNA synthetase), also known as Protein G7a, belongs to the class-I aminoacyl-tRNA synthetase family that includes the related proteins, LeuRS and IleRS. These proteins are large monomeric proteins and play a major role in catalyzing the aminoacylation of tRNA by their cognate amino acid. ValRS joins Val to tRNA(Val) at its synthetic active site. At its CP1 editing active site, ValRS hydrolyzes or deacylates tRNA(Thr) that is incorrectly joined to Val. ValRS forms aggregates with EF-1 (elongation factor 1) and, via this complex, catalyzes the aminoacylation of tRNA and its transfer to EF-1. In addition, ValRS may be regulated by PKC-dependent phosphorylation.

REFERENCES

1. Christ, D. and Winter, G. 2003. Identification of functional similarities between proteins using directed evolution. Proc. Natl. Acad. Sci. USA 100: 13202-13206.
2. Nordin, B.E. and Schimmel, P. 2003. Transiently misacylated tRNA is a primer for editing of misactivated adenylates by class I aminoacyl-tRNA synthetases. Biochemistry 42: 12989-12997.
3. Fukai, S., Nureki, O., Sekine, S., Shimada, A., Vassylyev, D.G. and Yokoyama, S. 2003. Mechanism of molecular interactions for tRNA(Val) recognition by valyl-tRNA synthetase. RNA 9: 100-111.
4. Jiang, S., Wolfe, C.L., Warrington, J.A. and Norcum, M.T. 2005. Three-dimensional reconstruction of the valyl-tRNA synthetase/elongation factor-1H complex and localization of the δ subunit. FEBS Lett. 579: 6049-6054.
5. Fukunaga, R. and Yokoyama, S. 2005. Structural basis for non-cognate amino acid discrimination by the valyl-tRNA synthetase editing domain. J. Biol. Chem. 280: 29937-29945.
6. Shitivelband, S. and Hou, Y.M. 2005. Breaking the stereo barrier of amino acid attachment to tRNA by a single nucleotide. J. Mol. Biol. 348: 513-521.
7. Fukunaga, R. and Yokoyama, S. 2005. Crystal structure of leucyl-tRNA synthetase from the archaeon *Pyrococcus horikoshii* reveals a novel editing domain orientation. J. Mol. Biol. 346: 57-71.

CHROMOSOMAL LOCATION

Genetic locus: VARS (human) mapping to 6p21.33.

PRODUCT

ValRS 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 ValRS shRNA Plasmid (h): sc-76887-SH and ValRS shRNA (h) Lentiviral Particles: sc-76887-V as alternate gene silencing products.

For independent verification of ValRS (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-76887A, sc-76887B and sc-76887C.

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

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

ValRS (D-7): sc-166674 is recommended as a control antibody for monitoring of ValRS 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 ValRS gene expression knockdown using RT-PCR Primer: ValRS (h)-PR: sc-76887-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.