SANTA CRUZ BIOTECHNOLOGY, INC.

IRAP siRNA (m): sc-146283

BACKGROUND

IRAP (Insulin-responsive aminopeptidase), also known as LNPEP (leucyl-cystinyl aminopeptidase), OTase (oxytocinase) or P-LAP (placental leucine aminopeptidase), is a 1,025 amino acid protein that is highly expressed in placenta, heart, kidney and small intestine and at lower levels in neuronal cells in brain, in skeletal muscle, spleen, liver, testes and colon. IRAP belongs to the peptidase M1 family and is thought to play a role in the degradation of hormones such as oxytocin, vasopressin and Angiotensin III. IRAP maintains homeostasis during pregnancy and may be involved in the inactivation of neuronal peptides in the brain. It is suggested that IRAP regulates the trafficking of the Insulin-responsive glucose transporter Glut4, thereby influencing glucose uptake in cells. IRAP interacts with Tankyrase-1 and Tankyrases-2, which are novel signaling targets of extracellular signal-regulated kinase (ERK) in the Golgi. Three isoforms exists due to alternative splicing.

REFERENCES

1. Chi, N.W. and Lodish, H.F. 2000. Tankyrase is a Golgi-associated mitogen-activated protein kinase substrate that interacts with IRAP in Glut4 vesicles. J. Biol. Chem. 275: 38437-38444.
2. Sbodio, J.I., et al. 2002. Tankyrase-2 oligomerizes with Tankyrase-1 and binds to both TRF1 (telomere-repeat-binding factor 1) and IRAP (insulin-responsive aminopeptidase). Biochem. J. 361: 451-459.
3. Yeh, T.Y., et al. 2007. Insulin-stimulated exocytosis of Glut4 is enhanced by IRAP and its partner tankyrase. Biochem. J. 402: 279-290.
4. Fernando, R.N., et al. 2007. Subcellular localization of Insulin-regulated membrane aminopeptidase, IRAP to vesicles in neurons. J. Neurochem. 102: 967-976.
5. Chai, S.Y., et al. 2008. Development of cognitive enhancers based on inhibition of Insulin-regulated aminopeptidase. BMC Neurosci. 9: S14.

CHROMOSOMAL LOCATION

Genetic locus: Lnpep (mouse) mapping to 17 A3.2.

PRODUCT

IRAP siRNA (m) is a target-specific 19-25 nt siRNA 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 IRAP shRNA Plasmid (m): sc-146283-SH and IRAP shRNA (m) Lentiviral Particles: sc-146283-V as alternate gene silencing products.

For independent verification of IRAP (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-146283A, sc-146283B and sc-146283C.

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 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

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

IRAP (F-5): sc-365300 is recommended as a control antibody for monitoring of IRAP 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 IRAP gene expression knockdown using RT-PCR Primer: IRAP (m)-PR: sc-146283-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.