SANTA CRUZ BIOTECHNOLOGY, INC.

Amisyn siRNA (h): sc-61968

BACKGROUND

Amisyn, also known as Syntaxin-binding protein 6 (STXBP6), is a mostly cytosolic protein related to Tomosyn which plays an important role in SNARE complex assembly. Amisyn contains a v-SNARE coiled coil homology domain that binds to Syntaxin 1A and weakly to Syntaxin 4. Three isoforms exist for Amisyn. Isoform 1 is the full length protein, isoform 2 has a different amino acid sequence between residues 204-210 and isoform 3 is missing amino acids 1-102 and contains a different sequence for amino acids 103-150. Amisyn lacks a transmembrane domain and therefore is unable to assemble into a functional, membrane-anchored SNARE complex. This suggests that Amisyn may instead be acting to maintain SNARE conformation and facilitate the binding of VAMP-2. Amisyn can inhibit exocytosis independent of Syntaxin binding.

REFERENCES

1. Scales, S.J., et al. 2002. Amisyn, a novel syntaxin-binding protein that may regulate SNARE complex assembly. J. Biol. Chem. 277: 28271-28279.
2. Widberg, C.H., et al. 2003. Tomosyn interacts with the t-SNAREs syntaxin4 and SNAP23.and plays a role in Insulin-stimulated GLUT4 translocation. J. Biol. Chem. 278: 35093-35101.
3. Gerst, J.E. 2003. SNARE regulators: matchmakers and matchbreakers. Biochim. Biophys. Acta 1641: 99-110.
4. Constable, J.R., et al. 2005. Amisyn regulates exocytosis and fusion pore stability by both syntaxin-dependent and syntaxin-independent mechanisms. J. Biol. Chem. 280: 31615-31623.
5. Maier, P., et al. 2006. Overexpression of MDR1 using a retroviral vector differentially regulates genes involved in detoxification and apoptosis and confers radioprotection. Radiat. Res. 166: 463-473.

CHROMOSOMAL LOCATION

Genetic locus: STXBP6 (human) mapping to 14q12.

PRODUCT

Amisyn 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 Amisyn shRNA Plasmid (h): sc-61968-SH and Amisyn shRNA (h) Lentiviral Particles: sc-61968-V as alternate gene silencing products.

For independent verification of Amisyn (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-61968A, sc-61968B and sc-61968C.

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.

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

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

Amisyn (F-9): sc-271959 is recommended as a control antibody for monitoring of Amisyn 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 Amisyn gene expression knockdown using RT-PCR Primer: Amisyn (h)-PR: sc-61968-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.