SANTA CRUZ BIOTECHNOLOGY, INC.

Amnionless siRNA (h): sc-60069

BACKGROUND

Megaloblastic anemia 1 (MGA1), also referred to as MGA1 Norwegian type or Imerslund-Grasbeck syndrome (I-GS), is a hereditary, recessive disorder caused by defects in the AMN gene. Patients suffering from MGA1 have a selective malabsorption of vitamin B12, causing impaired function of thymidine synthase which in turn interrupts DNA synthesis. Amnionless protein, encoded for by the AMN gene, is crucial for vitamin B12 absorption. It modulates a BMP (bone morphogenetic protein) signaling pathway and is therefore important for trunk mesoderm production during development. Amnionless is a membrane protein that interacts with cubulin and is primarily expressed in colon, kidney and small intestine. Shorter isoforms can also be detected in thymus, testis and peripheral blood leukocytes.

REFERENCES

1. Tomihara-Newberger, C., et al. 1999. The AMN gene product is required in extraembryonic tissues for the generation of middle primitive streak derivatives. Dev. Biol. 204: 34-54.
2. Kalantry, S., et al. 2001. The amnionless gene, essential for mouse gastrulation, encodes a visceral-endoderm-specific protein with an extracellular cysteine-rich domain. Nat. Genet. 27: 412-416.
3. Tanner, S.M., et al. 2003. Amnionless, essential for mouse gastrulation, is mutated in recessive hereditary megaloblastic anemia. Nat. Genet. 33: 426-429.
4. Strope, S., et al. 2004. Mouse amnionless, which is required for primitive streak assembly, mediates cell-surface localization and endocytic function of cubilin on visceral endoderm and kidney proximal tubules. Development 131: 4787-4795.
5. He, Q., et al. 2005. Amnionless function is required for cubilin brush-border expression and intrinsic factor-cobalamin (vitamin B_{12}) absorption *in vivo*. Blood 106: 1447-1453.
6. Gräsbeck, R. 2006. Imerslund-Gräsbeck syndrome (selective vitamin B_{12} malabsorption with proteinuria). Orphanet J. Rare Dis. 1: 17.

CHROMOSOMAL LOCATION

Genetic locus: AMN (human) mapping to 14q32.32.

PRODUCT

Amnionless siRNA (h) is a pool of 2 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 Amnionless shRNA Plasmid (h): sc-60069-SH and Amnionless shRNA (h) Lentiviral Particles: sc-60069-V as alternate gene silencing products.

For independent verification of Amnionless (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-60069A and sc-60069B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Amnionless shRNA Plasmid (h) is recommended for the inhibition of Amnionless 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

Amnionless (C-10): sc-365384 is recommended as a control antibody for monitoring of Amnionless 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 Amnionless gene expression knockdown using RT-PCR Primer: Amnionless (h)-PR: sc-60069-PR (20 µl, 434 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.