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Glucosidase II α siRNA (h): sc-41517

BACKGROUND

Trimming of glucoses from N-linked core glycans on newly synthesized glycoproteins occurs sequentially through the action of Glucosidases I and II in the endoplasmic reticulum (ER). Glucosidase II is an ER-localized enzyme that contains α and β subunits (Glucosidase II α and Glucosidase II β). The α and β subunits form a defined heterodimeric complex. Glucosidase II α is the catalytic core of the enzyme and can function independently of the β subunit. The sequence of Glucosidase II β encodes protein rich in glutamic and aspartic acid with a putative ER retention signal (HDEL) at the C terminus. The phosphorylated form of Glucosidase II β is localized in the plasma membrane and is highly expressed in FGF stimulated fibroblasts and epidermal carcinoma cells. Glucosidase II β was first purified from a human carcinoma cell line as a potential substrate for protein kinase C. Through the HDEL signal at the C-terminus, Glucosidase II β retains the complete complex in the ER.

REFERENCES

1. Shailubhai, K., et al. 1987. Purification and characterization of Glucosidase I involved in N-linked glycoprotein processing in bovine mammary gland. *Biochem. J.* 247: 555-562.
2. Saxena, S., et al. 1987. Purification and characterization of Glucosidase II involved in N-linked glycoprotein processing in bovine mammary gland. *Biochem. J.* 247: 563-570.
3. Trombetta, E.S., et al. 1996. Endoplasmic reticulum Glucosidase II is composed of a catalytic subunit, conserved from yeast to mammals, and a tightly bound noncatalytic HDEL-containing subunit. *J. Biol. Chem.* 271: 27509-27516.
4. Tremblé, K., et al. 2000. The α and β subunits are required for expression of catalytic activity in the heterodimeric Glucosidase II complex from human liver. *Glycobiology* 10: 493-502.
5. Trombetta, E.S., et al. 2001. Quaternary and domain structure of glycoprotein processing Glucosidase II. *Biochemistry* 40: 10717-10722.

CHROMOSOMAL LOCATION

Genetic locus: GANAB (human) mapping to 11q12.3.

PRODUCT

Glucosidase II α 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Glucosidase II α shRNA Plasmid (h): sc-41517-SH and Glucosidase II α shRNA (h) Lentiviral Particles: sc-41517-V as alternate gene silencing products.

For independent verification of Glucosidase II α (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-41517A, sc-41517B and sc-41517C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Glucosidase II α siRNA (h) is recommended for the inhibition of Glucosidase II α 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 μ M in 66 μ l. 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Glucosidase II α gene expression knockdown using RT-PCR Primer: Glucosidase II α (h)-PR: sc-41517-PR (20 μ l, 439 bp). 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.