SANTA CRUZ BIOTECHNOLOGY, INC.

p115 siRNA (m): sc-41283

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The Power to Question

BACKGROUND

The mammalian protein p115, known also as transcytosis-associated protein (TAP), tethering factor or vesicle docking protein (VDP), and its yeast homologue Uso1p have an essential role in membrane trafficking. p115 is phosphorylated in interphase but not in mitotic cells. Phosphorylated p115 is localized to the cytosol, whereas the unphosphorylated form is associated with membranes, mostly of the Golgi complex. Upon phosphorylation of p115 at Ser942, p115 is released from the membranes. In mammary glands, p115 synthesis is dependent of the stage of lactation. Both giantin and GM130 compete for binding to the C-terminal acidic domain of p115, and p115-giantin and p115-GM130 interactions mediate independent membrane tethering events. The amino terminal region of p115 is required for its localization to the Golgi. p115 is also expressed on transcytotic vesicles, where p115 is required for vesicle fusion with the target membrane and vesicular tubular clusters, which are involved in ER to Golgi transport. Rab1 recruits p115 to coat protein complex II (COPII) vesicles during budding from the endoplasmic reticulum, where it interacts with a select set of SNAREs. p115 is a general factor acting within the secretory and endocytic pathways to bind transport vesicles prior to membrane fusion.

REFERENCES

1. Barroso, M., et al. 1995. Transcytosis-associated protein (TAP)/p115 is a general fusion factor required for binding of vesicles to acceptor membranes. Proc. Natl. Acad. Sci. USA 92: 527-531.
2. Nelson, D.S., et al. 1998. The membrane transport factor TAP/p115 cycles between the Golgi and earlier secretory compartments and contains distinct domains required for its localization and function. J. Cell Biol. 143: 319-331.
3. Sohda, M., et al. 1998. Phosphorylation of the vesicle docking protein p115 regulates its association with the Golgi membrane. J. Biol. Chem. 273: 5385-5388.
4. Watanabe, A., et al. 2000. Development changes in the protein and mRNA content of a p115/transcytosis-associated protein in the bovine mammary gland. J. Endocrinol. 166: 319-327.
5. Linstedt, A.D., et al. 2000. Binding relationships of membrane tethering components. The giantin N-terminus and the GM130 N-terminus compete for binding to the p115 C-terminus. J. Biol. Chem. 275: 10196-10201.

CHROMOSOMAL LOCATION

Genetic locus: Uso1 (mouse) mapping to 5 E2.

PRODUCT

p115 siRNA (m) 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 p115 shRNA Plasmid (m): sc-41283-SH and p115 shRNA (m) Lentiviral Particles: sc-41283-V as alternate gene silencing products.

For independent verification of p115 (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-41283A, sc-41283B and sc-41283C.

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

p115 siRNA (m) is recommended for the inhibition of p115 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

p115 (H-11): sc-48363 is recommended as a control antibody for monitoring of p115 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 p115 gene expression knockdown using RT-PCR Primer: p115 (m)-PR: sc-41283-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.