

CHMP1A siRNA (h): sc-60367

BACKGROUND

The charged multivesicular body proteins or chromatin modifying proteins, commonly designated CHMPs, belong to the vacuolar sorting protein family and function as chromatin-modifying proteins. CHMP1-6 are all components of ESCRT (endosomal sorting complex required for transport) I, II or III complexes. These complexes are crucial for sorting endosomal articles into multivesicular bodies (MVBs), as well as required for the formation of these bodies. CHMP1 interacts with VPS4B and localizes to early endosomes. Two isoforms, encoded by distinct genes, exists for CHMP1. They are designated CHMP1A and CHMP1B. CHMP1 overexpression can lead to the dilation of endosomal compartments resulting in the disruption of normal distribution of endosomal markers.

REFERENCES

1. Stauffer, D.R., et al. 2001. CHMP1 is a novel nuclear matrix protein affecting chromatin structure and cell-cycle progression. *J. Cell Sci.* 114: 2383-2393.
2. Howard, T.L., et al. 2001. CHMP1 functions as a member of a newly defined family of vesicle trafficking proteins. *J. Cell Sci.* 114: 2395-2404.
3. Yang, K.S., et al. 2004. Molecular characterization of NbCHMP1 encoding a homolog of human CHMP1 in *Nicotiana benthamiana*. *Mol. Cells* 17: 255-261.
4. Reid, E., et al. 2004. The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B. *Hum. Mol. Genet.* 14: 19-38.
5. Horii, M., et al. 2006. CHMP7, a novel ESCRT-III-related protein, associates with CHMP4B and functions in the endosomal sorting pathway. *Biochem. J.* 400: 23-32.

CHROMOSOMAL LOCATION

Genetic locus: CHMP1A (human) mapping to 16q24.3.

PRODUCT

CHMP1A 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 CHMP1A shRNA Plasmid (h): sc-60367-SH and CHMP1A shRNA (h) Lentiviral Particles: sc-60367-V as alternate gene silencing products.

For independent verification of CHMP1A (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-60367A, sc-60367B and sc-60367C.

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 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

CHMP1A siRNA (h) is recommended for the inhibition of CHMP1A 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.

GENE EXPRESSION MONITORING

CHMP1A (B-5): sc-271617 is recommended as a control antibody for monitoring of CHMP1A 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 CHMP1A gene expression knockdown using RT-PCR Primer: CHMP1A (h)-PR: sc-60367-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.