

# CHMP4A siRNA (h): sc-60373

## 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) and are also required for the formation of these bodies. During HIV-1 infection, the virus uses the ESCRT-III complex to mediate budding and exocytosis of viral proteins via the association of CHMP4 and a protein recruited by HIV-1 p6, called PDCD61P, which is present in viral Gag assembly and budding. CHMP4 is strongly expressed in kidney, liver, heart and skeletal muscle and to a lesser degree in lung, brain and pancreas. Three CHMP4 isoforms, encoded by distinct genes, are designated CHMP4A, CHMP4B and CHMP4C.

## REFERENCES

1. Katoh, K., et al. 2003. CHMP4B is a major binding partner of the ALG-2-interacting protein Alix among the three CHMP4 isoforms. *Arch. Biochem. Biophys.* 421: 159-165.
2. von Schwedler, U.K., et al. 2003. The protein network of HIV budding. *Cell* 114: 701-713.
3. Katoh, K., et al. 2005. The penta-EF-hand protein ALG-2 interacts directly with the ESCRT-I component TSG101, and Ca<sup>2+</sup>-dependently co-localizes to aberrant endosomes with dominant-negative AAA ATPase SKD1/Vps4B. *Biochem. J.* 391: 677-685.

## CHROMOSOMAL LOCATION

Genetic locus: CHMP4A (human) mapping to 14q12.

## PRODUCT

CHMP4A 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CHMP4A shRNA Plasmid (h): sc-60373-SH and CHMP4A shRNA (h) Lentiviral Particles: sc-60373-V as alternate gene silencing products.

For independent verification of CHMP4A (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-60373A, sc-60373B and sc-60373C.

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

## APPLICATIONS

CHMP4A siRNA (h) is recommended for the inhibition of CHMP4A 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  $\mu$ M in 66  $\mu$ 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

CHMP4A (E-6): sc-514869 is recommended as a control antibody for monitoring of CHMP4A 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 CHMP4A gene expression knockdown using RT-PCR Primer: CHMP4A (h)-PR: sc-60373-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Petsalaki, E., et al. 2018. The ESCRT protein Chmp4c regulates mitotic spindle checkpoint signaling. *J. Cell Biol.* 217: 861-876.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.