

PHAX siRNA (h): sc-106785

BACKGROUND

Assembly of spliceosomal U snRNPs requires nuclear export of U snRNA precursors. PHAX (phosphorylated adaptor for RNA export, also designated resiniferatoxin-binding protein RBP-26) is the additional factor required for U snRNA export complex assembly *in vitro*. PHAX is present in sensory neuron cell bodies. *In vivo*, PHAX is required for U snRNA export but not for CRM1-mediated export in general. PHAX acts as an adaptor between the CBC/RNA complex and the CRM1/RanGTP proteins. PHAX is phosphorylated in the nucleus and then exported with RNA to the cytoplasm, where it is dephosphorylated. PHAX phosphorylation is essential for export complex assembly and its dephosphorylation causes export complex disassembly.

REFERENCES

1. Ninkina, N.N., et al. 1994. Molecular cloning of a resiniferatoxin-binding protein. *Brain Res. Mol. Brain Res.* 22: 39-48.
2. Fischer, U., et al. 1995. The HIV-1 Rev activation domain is a nuclear export signal that accesses an export pathway used by specific cellular RNAs. *Cell* 82: 475-483.
3. Fornerod, M., et al. 1997. CRM1 is an export receptor for leucine-rich nuclear export signals. *Cell* 90: 1051-1060.
4. Izaurralde, E., et al. 1997. The asymmetric distribution of the constituents of the Ran system is essential for transport into and out of the nucleus. *EMBO J.* 16: 6535-6547.
5. Ohno, M., et al. 2000. PHAX, a mediator of U snRNA nuclear export whose activity is regulated by phosphorylation. *Cell* 101: 187-198.

CHROMOSOMAL LOCATION

Genetic locus: PHAX (human) mapping to 5q23.2.

PRODUCT

PHAX 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 PHAX shRNA Plasmid (h): sc-106785-SH and PHAX shRNA (h) Lentiviral Particles: sc-106785-V as alternate gene silencing products.

For independent verification of PHAX (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-106785A, sc-106785B and sc-106785C.

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

PHAX siRNA (h) is recommended for the inhibition of PHAX 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

PHAX (F-1): sc-398147 is recommended as a control antibody for monitoring of PHAX 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 PHAX gene expression knockdown using RT-PCR Primer: PHAX (h)-PR: sc-106785-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Al-Lamki, R.S., et al. 2020. The efficacy of sunitinib treatment of renal cancer cells is associated with the protein PHAX *in vitro*. *Biology* 9: 74.

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.