

HRPAP20 siRNA (h): sc-62476

BACKGROUND

HRPAP20 (UPF0240 protein C6orf66, hormone-regulated proliferation-associated protein 20) is a 175 amino acid protein encoded by the human gene C6ORF66. HRPAP20 is a novel hormone-regulated, proliferation-associated protein. In tumor cell lines, constitutive HRPAP20 expression enhanced proliferation and suppressed apoptosis, characteristics frequently associated with malignant progression. Invasive breast cancer cell lines and human breast tumor specimens express elevated HRPAP20, which can increase malignant cell invasion in transfection experiments using such cell lines as MCF-7 and MDA-MB-231. Transfection with HRPAP20 will increase secretion of matrix metalloproteinase-9 (MMP-9). Conversely, knockdown of HRPAP20 with small interfering RNA will reduce invasion and inhibit secretion of MMP-9.

REFERENCES

1. Jones, J.L., et al. 2003. Primary breast myoepithelial cells exert an invasion-suppressor effect on breast cancer cells via paracrine down-regulation of MMP expression in fibroblasts and tumour cells. *J. Pathol.* 201: 562-572.
2. Karp, C.M., et al. 2004. Identification of HRPAP20: a novel phosphoprotein that enhances growth and survival in hormone-responsive tumor cells. *Cancer Res.* 64: 1016-1025.
3. Larkins, T.L., et al. 2006. Inhibition of cyclooxygenase-2 decreases breast cancer cell motility, invasion and matrix metalloproteinase expression. *BMC Cancer* 6: 181.
4. Jiang, W.G., et al. 2006. Expression of membrane type-1 matrix metalloproteinase, MT1-MMP in human breast cancer and its impact on invasiveness of breast cancer cells. *Int. J. Mol. Med.* 17: 583-590.
5. Byun, H.J., et al. 2006. A splice variant of CD99 increases motility and MMP-9 expression of human breast cancer cells through the AKT-, ERK-, and JNK-dependent AP-1 activation signaling pathways. *J. Biol. Chem.* 281: 34833-34847.
6. Tozlu-Kara, S., et al. 2007. Oligonucleotide microarray analysis of estrogen receptor α -positive postmenopausal breast carcinomas: identification of HRPAP20 and TIMELESS as outstanding candidate markers to predict the response to tamoxifen. *J. Mol. Endocrinol.* 39: 305-318.

CHROMOSOMAL LOCATION

Genetic locus: NDUFAF4 (human) mapping to 6q16.1.

PRODUCT

HRPAP20 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 HRPAP20 shRNA Plasmid (h): sc-62476-SH and HRPAP20 shRNA (h) Lentiviral Particles: sc-62476-V as alternate gene silencing products.

For independent verification of HRPAP20 (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-62476A, sc-62476B and sc-62476C.

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

HRPAP20 siRNA (h) is recommended for the inhibition of HRPAP20 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

HRPAP20 (G-10): sc-166928 is recommended as a control antibody for monitoring of HRPAP20 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 HRPAP20 gene expression knockdown using RT-PCR Primer: HRPAP20 (h)-PR: sc-62476-PR (20 μ l). 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.