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Prohibitin 2 siRNA (h): sc-45849

BACKGROUND

Prohibitin is an evolutionarily conserved protein that has antiproliferative activity. The gene encoding human Prohibitin maps to chromosome 17q21 and is ubiquitously expressed. Prohibitin is a post-synthetically modified protein that is localized in the inner membrane of mitochondria, where it regulates the cell cycle by blocking the transition between the G₁ and S phases, and on the plasma membrane of B cells, where it mediates B cell maturation. Prohibitin mRNA and protein levels are high in G₁, decline during the S phase, rise again in G₂ and decline in M phase, which suggests that Prohibitin controls the cell cycle by using both transcriptional and posttranslational mechanisms. Prohibitin is also a potential tumor suppressor protein that binds to retinoblastoma (Rb) and subsequently inhibits the activity of E2F family members in response to specific signaling cascades. Prohibitin 2 is a repressor of estrogen receptor activity and is required for somatic and germline differentiation in the larval gonad during embryonic development. Mutations in the Prohibitin genes are correlated with breast cancer development and/or progression in more than 80% of the cell lines analyzed.

REFERENCES

1. Sato, T., et al. 1992. The human Prohibitin gene located on chromosome 17q21 is mutated in sporadic breast cancer. *Cancer Res.* 52: 1643-1646.
2. Roskams, A.J., et al. 1993. Cell cycle activity and expression of Prohibitin mRNA. *J. Cell. Physiol.* 157: 289-295.
3. McClung, J.K., et al. 1995. Prohibitin: potential role in senescence, development, and tumor suppression. *Exp. Gerontol.* 30: 99-124.
4. Dell'Orco, R.T., et al. 1996. Prohibitin and the senescent phenotype. *Exp. Gerontol.* 31: 245-252.
5. Jupe, E.R., et al. 1996. Prohibitin in breast cancer cell lines: loss of antiproliferative activity is linked to 3' untranslated region mutations. *Cell Growth Differ.* 7: 871-888.
6. Wang, S., et al. 1999. Prohibitin, a potential tumor suppressor, interacts with RB and regulates E2F function. *Oncogene* 18: 3501-3510.

CHROMOSOMAL LOCATION

Genetic locus: PHB2 (human) mapping to 12p13.31.

PRODUCT

Prohibitin 2 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 Prohibitin 2 shRNA Plasmid (h): sc-45849-SH and Prohibitin 2 shRNA (h) Lentiviral Particles: sc-45849-V as alternate gene silencing products.

For independent verification of Prohibitin 2 (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-45849A, sc-45849B and sc-45849C.

RESEARCH USE

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

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

Prohibitin 2 siRNA (h) is recommended for the inhibition of Prohibitin 2 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

Prohibitin 2 (A-2): sc-133094 is recommended as a control antibody for monitoring of Prohibitin 2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Prohibitin 2 gene expression knockdown using RT-PCR Primer: Prohibitin 2 (h)-PR: sc-45849-PR (20 μ l, 520 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.