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# Prohibitin 2 shRNA (h) Lentiviral Particles: sc-45849-V

## 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 G<sub>1</sub> 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 G<sub>1</sub>, decline during the S phase, rise again in G<sub>2</sub> 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 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Prohibitin 2 siRNA (h): sc-45849 and Prohibitin 2 shRNA Plasmid (h): sc-45849-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

Prohibitin 2 shRNA (h) Lentiviral Particles is recommended for the inhibition of Prohibitin 2 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10<sup>6</sup> infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 µl, 520 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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