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- Expressversand

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# ER $\alpha$ shRNA (h2) Lentiviral Particles: sc-44204-V

## BACKGROUND

Estrogen receptors (ER) are members of the steroid/thyroid hormone receptor superfamily of ligand-activated transcription factors. Estrogen receptors, including ER $\alpha$  and ER $\beta$ , contain DNA binding and ligand binding domains and are critically involved in regulating the normal function of reproductive tissues. They are located in the nucleus, though some estrogen receptors associate with the cell surface membrane and can be rapidly activated by exposure of cells to estrogen. ER $\alpha$  and ER $\beta$  have been shown to be differentially activated by various ligands. Receptor-ligand interactions trigger a cascade of events, including dissociation from heat shock proteins, receptor dimerization, phosphorylation and the association of the hormone activated receptor with specific regulatory elements in target genes. Evidence suggests that ER $\alpha$  and ER $\beta$  may be regulated by distinct mechanisms even though they share many functional characteristics.

## REFERENCES

- Mason, B.H., et al. 1983. Progesterone and estrogen receptors as prognostic variables in breast cancer. *Cancer Res.* 43: 2985-2990.
- Evans, R.M. 1988. The steroid and thyroid hormone receptor superfamily. *Science* 240: 889-895.
- Danielian, P.S., et al. 1992. Identification of a conserved region required for hormone dependent transcriptional activation by steroid hormone receptors. *EMBO J.* 11: 1025-1033.
- Kliwer, S.A., et al. 1992. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signaling. *Nature* 355: 446-449.
- Arnold, S.F., et al. 1995. Phosphorylation of the human estrogen receptor on Tyrosine 537 *in vivo* and by Src family tyrosine kinases *in vitro*. *Mol. Endocrinol.* 9: 24-33.
- Mosselman, S., et al. 1996. ER $\beta$ : identification and characterization of a novel human estrogen receptor. *FEBS Lett.* 392: 49-53.

## CHROMOSOMAL LOCATION

Genetic locus: ESR1 (human) mapping to 6q25.1.

## PRODUCT

ER $\alpha$  shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 ER $\alpha$  siRNA (h2): sc-44204 and ER $\alpha$  shRNA Plasmid (h2): sc-44204-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

ER $\alpha$  shRNA (h2) Lentiviral Particles is recommended for the inhibition of ER $\alpha$  expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

ER $\alpha$  (F-10): sc-8002 is recommended as a control antibody for monitoring of ER $\alpha$  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<sup>™</sup> compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker<sup>™</sup> 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<sup>™</sup> Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ER $\alpha$  gene expression knockdown using RT-PCR Primer: ER $\alpha$  (h2)-PR: sc-44204-PR (20  $\mu$ l, 501 bp). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  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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