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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

ARA54 shRNA (h) Lentiviral Particles: sc-43618-V

BACKGROUND

Androgens exhibit a wide range of effects on the development, maintenance and regulation of male phenotype and reproductive physiology in males. The androgen receptor (AR) is a member of the steroid superfamily of ligand-dependent transcription factors. ARs bind the two biologically active androgens, testosterone (T) and dihydrotestosterone (DHT). ARA54 contains a RING finger and functions as an AR coactivator. Testis, thymus, spleen, colon, prostate and uterus express ARA54 at high levels. ARA160 associates with an ATP-dependent chromatin remodeling factor known as the SNF/SWI complex. The FXXLF motif present in AR coactivators mediates their interaction with AR but not their transcription-related activity. The genes encoding human ARA54 and ARA160 map to chromosomes 5q31.3 and 3p21-p12, respectively.

REFERENCES

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- Imperato-McGinley, J., et al. 1974. Steroid 5A-reductase deficiency in man: an inherited form of male pseudohermaphroditism. *Science* 186: 1213-1215.
- Garcia, J.A., et al. 1992. Cloning and chromosomal mapping of a human immunodeficiency virus 1 "TATA" element modulatory factor. *Proc. Natl. Acad. Sci. USA* 89: 9372-9376.
- Zhou, Z.X., et al. 1994. The androgen receptor: an overview. *Recent Prog. Horm. Res.* 49: 249-274.
- Kang, H.Y., et al. 1999. Cloning and characterization of human prostate coactivator ARA54, a novel protein that associates with the androgen receptor. *J. Biol. Chem.* 274: 8570-8576.

CHROMOSOMAL LOCATION

Genetic locus: RNF14 (human) mapping to 5q31.3.

PRODUCT

ARA54 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 μ l frozen stock containing 1.0×10^6 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 ARA54 siRNA (h): sc-43618 and ARA54 shRNA Plasmid (h): sc-43618-SH as alternate gene silencing products.

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.

APPLICATIONS

ARA54 shRNA (h) Lentiviral Particles is recommended for the inhibition of ARA54 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

ARA54 (B-10): sc-376701 is recommended as a control antibody for monitoring of ARA54 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 ARA54 gene expression knockdown using RT-PCR Primer: ARA54 (h)-PR: sc-43618-PR (20 μ l). 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.

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.

PROTOCOLS

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