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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) 

ARD1 shRNA (m) Lentiviral Particles: sc-44714-V

BACKGROUND

The ARD1 subfamily of proteins belongs to the larger acetyltransferase family. N-terminal acetyltransferase complex ARD1, also designated Te2, forms a complex with NARG1, displaying N-terminal acetyltransferase activity. Without NARG1, ARD1 promotes hypoxia-inducible factor-1 α (HIF-1 α) degradation by displaying internal acetyltransferase activity towards HIF-1 α . This ubiquitously expressed protein, which is mainly cytoplasmic, is cleaved by caspases during apoptosis. ARD1 interacts with the ribosome, NARG1 and HIF-1 α . In its binding to HIF-1 α , ARD1 acts as a protein acetyltransferase by regulating its stability. In many cell lines, ARD1 is downregulated in response to hypoxia. ARD1 is expressed throughout the developing brain.

REFERENCES

1. Jeong, J.W., et al. 2002. Regulation and destabilization of HIF-1 α by ARD1-mediated acetylation. *Cell* 111: 709-720.
2. Sugiura, N., et al. 2003. An evolutionarily conserved N-terminal acetyltransferase complex associated with neuronal development. *J. Biol. Chem.* 278: 40113-40120.
3. Carninci, P., et al. 2005. The transcriptional landscape of the mammalian genome. *Science* 309: 1559-1563.
4. Fisher, T.S., et al. 2005. Analysis of ARD1 function in hypoxia response using retroviral RNA interference. *J. Biol. Chem.* 280: 17749-17757.
5. Vichi, A., et al. 2005. E3 ubiquitin ligase activity of the trifunctional ARD1 (ADP-ribosylation factor domain protein 1). *Proc. Natl. Acad. Sci. USA* 102: 1945-1950.
6. Lee, K.H., et al. 2006. Differential responses of two degradation domains of HIF-1 α to hypoxia and iron deficiency. *Biochimie* 88: 163-169.

CHROMOSOMAL LOCATION

Genetic locus: *Ard1* (mouse) mapping to X A7.3.

PRODUCT

ARD1 shRNA (m) 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 ARD1 siRNA (m): sc-44714 and ARD1 shRNA Plasmid (m): sc-44714-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.

PROTOCOLS

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

APPLICATIONS

ARD1 shRNA (m) Lentiviral Particles is recommended for the inhibition of ARD1 expression in mouse 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

ARD1 (E-16): sc-33256 is recommended as a control antibody for monitoring of ARD1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ARD1 gene expression knockdown using RT-PCR Primer: ARD1 (m)-PR: sc-44714-PR (20 μ l). 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.