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# ASH2L shRNA (h) Lentiviral Particles: sc-43556-V

## BACKGROUND

The human ASH2L gene encodes a 628 amino acid protein known as ASH2L1, or isoform 1, which contains a nuclear localization signal and PHD finger motif, suggesting that the gene product functions as a transcription regulator. Alternative splicing results in a shorter isoform 2, designated ASH2L2, which is missing the first 94 amino acid residues found in ASH2L1. Human ASH2L proteins are 60% homologous to *Drosophila ash2*, which positively regulates expression of certain genes in early development, and contain similar, but not identical, domains, including a zinc finger motif. ASH2L is highly expressed in fetal liver, testis and leukemia cell lines with erythroid and megakaryocytic potential, such as K562, Hel and Dami. Differentiation inducers (e.g. phorbol ester and hemin) cause different expression patterns in these cells lines, suggesting that ASH2L plays a role in hematopoiesis and is associated with particular types of leukemia.

## REFERENCES

- Ikegawa, S., et al. 1999. Cloning and characterization of ASH2L and Ash2l, human and mouse homologs of the *Drosophila ash2* gene. *Cytogenet. Cell Genet.* 84: 167-172.
- Wang, J., et al. 2001. ASH2L: alternative splicing and downregulation during induced megakaryocytic differentiation of multipotential leukemia cell lines. *J. Mol. Med.* 79: 399-405.
- Amoros, M., et al. 2002. The ash2 gene is involved in *Drosophila* wing development. *Int. J. Dev. Biol.* 46: 321-324.
- Wysocka, J., et al. 2003. Human Sin3 deacetylase and trithorax-related Set1/Ash2 histone H3-K4 methyltransferase are tethered together selectively by the cell-proliferation factor HCF-1. *Genes Dev.* 17: 896-911.
- Beltran, S., et al. 2003. Transcriptional network controlled by the trithorax-group gene ash2 in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* 100: 3293-3298.
- Angulo, M., et al. 2004. Activation and repression activities of ash2 in *Drosophila* wing imaginal discs. *Development* 131: 4943-4953.

## CHROMOSOMAL LOCATION

Genetic locus: ASH2L (human) mapping to 8p11.23.

## PRODUCT

ASH2L 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  $\mu$ 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 ASH2L siRNA (h): sc-43556 and ASH2L shRNA Plasmid (h): sc-43556-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

ASH2L shRNA (h) Lentiviral Particles is recommended for the inhibition of ASH2L 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

ASH2L (H-300): sc-367190 is recommended as a control antibody for monitoring of ASH2L 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ASH2L gene expression knockdown using RT-PCR Primer: ASH2L (h)-PR: sc-43556-PR (20  $\mu$ l, 497 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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