

# ASA siRNA (h): sc-72542

## BACKGROUND

ASA (arylsulfatase A), also known as Cerebroside-sulfatase, ARSA or MLD, is a 507 amino acid lysosomal protein that belongs to the sulfatase family. Functioning as a homodimer at a neutral pH and as a homo-octamer at an acidic pH, ASA uses magnesium as a cofactor to catalyze the H<sub>2</sub>O-dependent hydrolysis of cerebroside 3-sulfate to cerebroside and sulfate. Defects in the gene encoding ASA are a cause of metachromatic leukodystrophy (MLD), an intralysosomal storage disease that is characterized by ataxias, dementia, seizures, spastic tetraparesis and, ultimately, death. Additionally, defects in ASA activity are associated with multiple sulfatase deficiency (MSD), a disorder that results in decreased activity of all known sulfatases and is generally characterized by metachromatic leukodystrophy, mucopolysaccharidosis, chondrodysplasia punctata, hydrocephalus, ichthyosis, neurologic deterioration and developmental delay.

## REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607574. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Jean, S., et al. 2006. Ethanol decreases rat hepatic arylsulfatase A activity levels. *Alcohol. Clin. Exp. Res.* 30: 1950-1955.
3. Jimenez, I., et al. 2006. Carbohydrate affinity chromatography indicates that arylsulfatase-A from capacitated boar sperm has mannose and N-acetylglucosamine/sialic acid residues. *Arch. Androl.* 52: 455-462.
4. Biffi, A., et al. 2006. Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice. *J. Clin. Invest.* 116: 3070-3082.
5. Sevin, C., et al. 2007. Partial cure of established disease in an animal model of metachromatic leukodystrophy after intracerebral adeno-associated virus-mediated gene transfer. *Gene Ther.* 14: 405-414.
6. Consiglio, A., et al. 2007. Metabolic correction in oligodendrocytes derived from metachromatic leukodystrophy mouse model by using encapsulated recombinant myoblasts. *J. Neurol. Sci.* 255: 7-16.

## CHROMOSOMAL LOCATION

Genetic locus: ARSA (human) mapping to 22q13.33.

## PRODUCT

ASA siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ASA shRNA Plasmid (h): sc-72542-SH and ASA shRNA (h) Lentiviral Particles: sc-72542-V as alternate gene silencing products.

For independent verification of ASA (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72542A, sc-72542B and sc-72542C.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

ASA siRNA (h) is recommended for the inhibition of ASA expression in human cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μM in 66 μl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

ASA (H-5): sc-365176 is recommended as a control antibody for monitoring of ASA 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ASA gene expression knockdown using RT-PCR Primer: ASA (h)-PR: sc-72542-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.