Product Description SALSA® MLPA® Probemix P441-A2 SACS

To be used with the MLPA General Protocol.

Version A2. Compared to version A1, four reference probes have been replaced. For complete product history see page 5.

Catalogue numbers:
- **P441-025R:** SALSA MLPA Probemix P441 SACS, 25 reactions.
- **P441-050R:** SALSA MLPA Probemix P441 SACS, 50 reactions.
- **P441-100R:** SALSA MLPA Probemix P441 SACS, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

General information: The SALSA MLPA Probemix P441 SACS is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SACS* gene, which is associated with autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS).

ARSACS is a neurodegenerative disorder, characterised by early-onset progressive cerebellar ataxia with spasticity and peripheral neuropathy. The classic form of ARSACS is often displayed in early childhood, leading to delayed walking in young toddlers, while individuals with disease onset in teenage or early-adult years are also being described more recently. ARSACS is caused by mutations in the *SACS* gene, which encodes the sacsin protein believed to integrate the ubiquitin-proteasome system and Hsp70 chaperone machinery and implicated in the processing of ataxin-1. Sacsin is most highly expressed in large neurons, including cerebellar Purkinje cells

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1255/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Exon numbering: The *SACS* exon numbering used in this P441-A2 SACS product description is the exon numbering from the RefSeq transcript NM_014363.6, which is identical to the NG_012342.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 03/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P441-A2 SACS contains 28 MLPA probes with amplification products between 130 and 458 nucleotides (nt). This includes 18 probes for the *SACS* gene, one probe for exons 4, 8, and 9, three probes for exon 10, and two probes for the rest of the exons. Furthermore, one flanking probe is included to facilitate the determination of the extent of a deletion/duplication. In addition, nine reference probes are included that detect autosomal chromosomal locations. Complete probe

sequences and the identity of the genes detected by the reference probes are available online (www.mlpa.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens: Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples: A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from unrelated individuals who are from families without a history of autosomal recessive spastic ataxia of Charlevoix-Saguenay. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

Data analysis: Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results: The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *SACS* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P441 SACS.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results: Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***SACS* mutation database:** https://databases.lovd.nl/shared/genes/SACS. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *SACS* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P441-A2 SACS

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*SACS*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 13867-L15385	16p13	
136 *	Reference probe 20515-L28105	1q31	
142	**SACS probe** 17640-L21700		**Exon 5**
148	**SACS probe** 17641-L23550		**Exon 3**
154	**SACS probe** 17642-L21702		**Exon 10**
160	**SACS probe** 17643-L23551		**Exon 7**
166	**SACS probe** 17644-L21704		**Exon 3**
172	**SACS probe** 17645-L21705		**Exon 2**
179	**SACS probe** 17646-L21706		**Exon 10**
184	**SACS probe** 17647-L21707		**Exon 8**
202	**SACS probe** 17650-L21710		**Exon 7**
220 *	Reference probe 21710-L30368	15q21	
229	**SACS probe** 17652-L21712		**Exon 6**
256	**SACS probe** 17905-L22207		**Exon 9**
265	**SACS probe** 17657-L21717		**Exon 1**
274	**SACS probe** 17658-L21718		**Exon 6**
283	Reference probe 04404-L02610	14q22	
292	**SACS probe** 17659-L21719		**Exon 4**
310	Reference probe 09245-L09435	7q22	
328 *	Reference probe 19605-L29238	8q13	
339	**SACS probe** 17661-L21721		**Exon 10**
345	**SACS probe** 17662-L21722		**Exon 1**
382	**SACS probe** 17663-L21723		**Exon 5**
391 ¬	SGCG probe 17664-L23553		Downstream
407	Reference probe 14840-L23554	10q26	
436	**SACS probe** 17665-L21725		**Exon 2**
445 *	Reference probe 20720-L28598	2p22	
458	Reference probe 14844-L21137	18q21	

a) See above section on exon numbering for more information.

* New in version A2.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2. *SACS* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	SACS exon[a]	Ligation site NM_014363.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*590-592 (Exon 2)*		
265	17657-L21717	Exon 1	17-18	GGCTGCGGGACA-GTCACGCTGGGA	0.5 kb
345	17662-L21722	Exon 1	421 nt after exon 1	GTGGGGTCCAGA-GTCTTATTGAAC	21.5 kb
436	17665-L21725	Exon 2	140-141	CAGAAGAAAGGC-TCCTACCAAAGA	0.3 kb
172	17645-L21705	Exon 2	411-412	ACACACGACAAT-GTTTCCTGACAT	35.5 kb
148	17641-L23550	Exon 3	636 nt before exon 3	CCTAGAAATGCC-TAGTTCTGCATT	0.7 kb
166	17644-L21704	Exon 3	713-714	TCTTCGCGGAGA-CTGGCTTCCCGG	5.0 kb
292	17659-L21719	Exon 4	881 nt after exon 4, reverse	CCACGCTTGTCT-TCCTCTGGCCCT	1.7 kb
142	17640-L21700	Exon 5	860-861	GTCGATTTGGTC-AGACAACGCCAC	0.1 kb
382	17663-L21723	Exon 5	927-928	AGGAGGACAGAT-TCTTAAGGTGGG	3.2 kb
229	17652-L21712	Exon 6	1020-1021	AGAGACTCTTTG-GTCAAAAGATAT	0.2 kb
274	17658-L21718	Exon 6	157 nt after exon 6	TTTGGTGGGAAG-GTGTCTCCACCA	6.5 kb
160	17643-L23551	Exon 7	26 nt before exon 7	TCTTTTTTGAAT-TCCTGCCTGCGC	0.1 kb
202	17650-L21710	Exon 7	1138-1139	AAAAAGGATGAT-CCTCTGAAGGTC	3.7 kb
184	17647-L21707	Exon 8	2523-2524	GGGCTGTGCTGA-AGAAAAGCTTCA	0.8 kb
256	17905-L22207	Exon 9	2730-2731	GGATAACTTGAA-ACCTCACCTTGT	15.0 kb
179	17646-L21706	Exon 10	5596-5597	ACAGCAGATATT-TATTCTCTTGTG	3.5 kb
154	17642-L21702	Exon 10	9112-9113	TCAGCTCACAAG-AACCAAGATATT	6.7 kb
339	17661-L21721	Exon 10	198 nt after exon 10	TTTAGGTGCTCA-TTGATTTAGTTG	124.8 kb
		stop codon	*14327-14329 (Exon 10)*		
391 ¬	17664-L23553	*SGCG*		TTAGCTCTTACA-ATTTGGATTCTT	

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Related SALSA MLPA probemixes

P316 Recessive Ataxias — Contains probes for the *SETX*, *APTX*, and *FXN* genes involved in multiple recessive ataxias.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P441 SACS

- D'Amore A et al. (2018). Next generation molecular diagnosis of hereditary spastic paraplegias: an Italian cross-sectional study. *Front Neurol*, 9, 981.

P441 Product history	
Version	*Modification*
A2	Four reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version A2-01 — 23 April 2020 (02P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *SACS* gene updated according to new version of the NM_ reference sequence. *Version 02 – 01 May 2017 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). - Related probemix added on page 1. - Updated link for "Database of Genomic Variants". - Various minor textual and layout changes. *Version 01 (50)* - Not applicable, new document.

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