Product Description SALSA® MLPA® Probemix P459-A2 SERPINA1

To be used with the MLPA General Protocol.

Version A2. Compared to previous version A1, one reference probe has been replaced. For complete product history see page 5.

Catalogue numbers:
- **P459-025R:** SALSA MLPA Probemix P459 SERPINA1, 25 reactions.
- **P459-050R:** SALSA MLPA Probemix P459 SERPINA1, 50 reactions.
- **P459-100R:** SALSA MLPA Probemix P459 SERPINA1, 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 P459 SERPINA1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SERPINA1* gene, which is associated with Alpha-1 antitrypsin deficiency (AATD).

AATD is an autosomal recessive disorder and is characterised by an increased risk for chronic obstructive pulmonary disease (COPD) in adults and liver disease in children and adults which, amongst others, may result in cirrhosis and panniculitis. Defects in the *SERPINA1* gene on chromosome 14 are the main cause of AATD. The protein encoded by this gene is alpha-1-antitrypsin (AAT), also known as protease inhibitor (PI). The most important role of AAT is inhibiting neutrophil elastase, a protease that degrades elastin in the alveolar walls as well as other structural proteins in a variety of tissues. Defects in the *SERPINA1* gene can lead to tissue damage due to uncontrolled elastase activity.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1519/.

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 *SERPINA1* exon numbering used in this P459-A2 SERPINA1 product description is the exon numbering from the RefSeq transcript NM_001127701.1 (transcript variant 5), which is identical to the NG_008290.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 06/2019. 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 P459-A2 SERPINA1 contains 21 MLPA probes with amplification products between 142 and 301 nucleotides (nt). This includes nine probes for the *SERPINA1* gene, one probe for every exon, two probes for exon 4, and one probe for an additional exon which is only present in transcript variant 1 (NM_000295.4 sequence). In addition, 12 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 congenital lung disorders. 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

Copy number status	Dosage quotient
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 *SERPINA1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P459 SERPINA1.
- 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.

SERPINA1 **mutation database:** https://databases.lovd.nl/shared/genes/SERPINA1. 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 *SERPINA1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P459-A2 SERPINA1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	SERPINA1
64-105	Control fragments – see table in probemix content section for more information		
142	Reference probe 13872-L15390	7p12	
151	**SERPINA1 probe** S1118-L28907		**Exon 5**
154	Reference probe 12416-L13417	22q12	
160	**SERPINA1 probe** 20181-L28416		**Intron 2**
171 *	Reference probe 19185-L25211	3q23	
178 Ж	**SERPINA1 probe** 20182-SP0929-L27459		**Exon 4**
183	Reference probe 19757-L26540	9q34	
190	**SERPINA1 probe** 20183-L27460		**Exon 7**
196	Reference probe 10484-L20012	11q13	
202	**SERPINA1 probe** 20184-L27461		**Exon 2**
210	Reference probe 03559-L04800	3p22	
220 Ж	**SERPINA1 probe** 20185-SP0930-L27462		**Exon 4**
228	Reference probe 16505-L18966	5q14	
238	**SERPINA1 probe** 20186-L27463		**Exon 3**
247	Reference probe 14971-L16707	6q22	
255	Reference probe 09899-L10312	16p13	
264	Reference probe 11695-L12466	17q25	
274	**SERPINA1 probe** 20188-L27465		**Exon 1**
283	Reference probe 06754-L06358	8q12	
292	**SERPINA1 probe** 20189-L27466		**Exon 6**
301	Reference probe 07601-L07286	15q26	

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

* New in version A2.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

Table 2. *SERPINA1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	SERPINA1 exon[a]	Ligation site NM_001127701.1	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*574-576 (Exon 4)*		
274	20188-L27465	Exon 1	197-198	TGCCTGCCAGAA-GAGACAGAGCTT	1.6 kb
202	20184-L27461	Exon 2	365-366	AAGGATTCTGCA-GTGAGAGCAGAG	0.2 kb
160 +	20181-L28416	Intron 2	43 nt before exon 3 (NM_000295.4; 110-111)	TTAGCCCCTGTT-TGCTCCTCCGAT	0.1 kb
238	20186-L27463	Exon 3	513-514	AATACGGACGAG-GACAGGGCCCTG	5.7 kb
220 Ж	20185-SP0930-L27462	Exon 4	850-851 and 889-890	CCCTGGGGACCA-39 nt spanning oligo-TCAACCTCACGG	0.3 kb
178 Ж	20182-SP0929-L27459	Exon 4	1176-1177 and 1216-1217	AAGGAGCTTGAC-40 nt spanning oligo-AAGGTAAGGTTG	1.7 kb
151	S1118-L28907	Exon 5	1402-1403	CCGCCATCTTCT-TCCTGCCTGATG	1.3 kb
292	20189-L27466	Exon 6	1509-1510	AGCTTACATTTA-CCCAAACTGTCC	1.2 kb
190 #	20183-L27460	Exon 7	1837-1838	AATAACTGCCTC-TCGCTCCTCAAC	
		stop codon	*1828-1830 (Exon 7)*		

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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
+ This probe sequence is located in exon 1 of the NM_000295.4 sequence, which represents transcript variant 1.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

Related SALSA MLPA probemixes

P227 SERPINC1	Contains probes for the *SERPINC1* gene, involved in antithrombin deficiency.
P243 SERPING1	Contains probes for the *SERPING1* gene, involved in hereditary angioedema (HAE).

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 P459 SERPINA1

- Carpagnano GE et al. (2017). A new SERPINA-1 missense mutation associated with alpha-1 antitrypsin deficiency and bronchiectasis. *Lung*, *195*(5), 679-682.
- Speevak MD et al. (2019). An unusual case of alpha-1-antitrypsin deficiency: SZ/Z. *Clin Biochem*, *64*, 49-52.

P459 Product history	
Version	*Modification*
A2	One reference probe has been replaced.
A1	First release.

Implemented changes in the product description

Version A2-01 — 10 July 2019 (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).
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 01 – 06 October 2015 (55)
- Not applicable, new document.

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