Product Description
SALSA® MLPA® Probemix P227-B4 SERPINC1

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, three reference probes have been replaced.
For complete product history see page 6.

Catalogue numbers:
- **P227-025R:** SALSA MLPA Probemix P227 SERPINC1, 25 reactions.
- **P227-050R:** SALSA MLPA Probemix P227 SERPINC1, 50 reactions.
- **P227-100R:** SALSA MLPA Probemix P227 SERPINC1, 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.mrcholland.com).

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

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 P227 SERPINC1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SERPINC1* gene, which is associated with antithrombin deficiency.

Antithrombin deficiency is a rare hereditary disorder that generally comes to light when a patient suffers from recurrent venous thrombosis and pulmonary embolism. In renal failure (especially nephrotic syndrome) antithrombin is lost in the urine. This leads to a higher activity of Factor II and Factor X and an increased tendency to thrombosis. Antithrombin (III) deficiency is mainly caused by a defect in the *SERPINC1* gene.

The *SERPINC1* gene (7 exons) spans ~13.5 kb of genomic DNA, and is located on chromosome 1q25.1, ~173 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK284694/.

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 *SERPINC1* exon numbering used in this P227-B4 SERPINC1 product description is the exon numbering from the exon numbering from the NG_012462.1 sequence. The exon numbering of the NM_000488.4 sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG or NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P227-B4 SERPINC1 contains 17 MLPA probes with amplification products between 136 and 328 nucleotides (nt). This P227-B4 includes one probe for each exon of the *SERPINC1* gene. In addition, 10 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.mrcholland.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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 different unrelated individuals who are from families without a history of Antithrombin deficiency. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples 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.mrcholland.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 final ratio (FR) 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 FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also 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.
- 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: a lower injection voltage or a shorter injection time, 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 *SERPINC1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P227-B4 SERPINC1.

- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

***SERPINC1* mutation databases**

https://databases.lovd.nl/shared/genes/*SERPINC1*. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. 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 SNVs and unusual results (e.g., a duplication of *SERPINC1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P227-B4 SERPINC1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SERPINC1*
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 09067-L09236	19p	
148	**SERPINC1** probe 07328-L06965		**Exon 2**
160 *	Reference probe 19366-L25759	3p	
172	**SERPINC1** probe 07331-L06968		**Exon 5**
184	Reference probe 09286-L09517	11q	
196	**SERPINC1** probe 07329-L06966		**Exon 3**
209	Reference probe 14108-L16359	8p	
220	**SERPINC1** probe 07333-L06970		**Exon 7**
233	Reference probe 16528-L00940	17p	
247	**SERPINC1** probe 07330-L06967		**Exon 4**
256 *	Reference probe 19310-L25537	7q	
268	Reference probe 16225-L18478	16q	
283	**SERPINC1** probe 07327-L06964		**Exon 1**
292	Reference probe 13325-L14751	18q	
301 *	Reference probe 21215-L29590	9p	
319	**SERPINC1** probe 07332-L06969		**Exon 6**
328	Reference probe 17044-L20107	10p	

[a] See section Exon numbering on page 1 for more information.

* New in version B4.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P227-B4 probes arranged according to chromosomal location

Tabel 2. *SERPINC1* gene

Length (nt)	SALSA MLPA probe	*SERPINC1* exon[a]	Ligation site NM_000488.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*69-71 (exon 1)*		
283	07327-L06964	Exon 1	105-106	TAACCTCTGGAA-AAAGGTAAGAGG	2.6 kb
148	07328-L06965	Exon 2	377-378	GATTCCAAGAAT-GACAATGATAAC	2.8 kb
196	07329-L06966	Exon 3	636-637	TCAATGAGACCT-ACCAGGACATCA	1.1 kb
247	07330-L06967	Exon 4	788-789	GAAGCCATCAAT-GAGCTCACTGTT	1.2 kb
172	07331-L06968	Exon 5	1155-1156	ACGGCTTCAGTT-TGAAGGAGCAGC	2.1 kb
319	07332-L06969	Exon 6	1266-1267	TCTCAGATGCAT-TCCATAAGGCAT	3.4 kb
220	07333-L06970	Exon 7	1327-1328	AAGTACCGCTGT-TGTGATTGCTGG	
		stop codon	*1461-1463 (exon 7)*		

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

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 P227 SERPINC1

- Caspers M et al. (2012). Deficiencies of antithrombin, protein C and protein S - Practical experience in genetic analysis of a large patient cohort. *Thromb Haemost*. 108:247-57.
- De la Morena-Barrio B et al. (2019). Incidence and features of thrombosis in children with inherited antithrombin deficiency. *Haematologica*. 104(12): 2512–2518.
- Kato Io et al. (2014). A complex genomic abnormality found in a patient with antithrombin deficiency and autoimmune disease-like symptoms. *Int J Lab Hematol*. 100.2: 200-205.
- Lee ST et al. (2008). Detection of large deletion mutations in the SERPINC1 gene causing hereditary antithrombin deficiency by multiplex ligation-dependent probe amplification (MLPA). *J Thromb Haemost*. 6:701-3.
- Sekiya A et al. (2017). Causative genetic mutations for antithrombin deficiency and their clinical background among Japanese patients. *Int J Hematol*. 105.3: 287-294.
- Sekiya A et al. (2011). Two case reports of inherited antithrombin deficiency: a novel frameshift mutation and a large deletion including all seven exons detected using two methods. *Int J Hematol*. 93:216-9.
- Zeng W et al. (2015). Genetic analysis should be included in clinical practice when screening for antithrombin deficiency. *Thromb Haemost*. 113(2):262-71.

P227 product history	
Version	*Modification*
B4	Three reference probes have been replaced.
B3	Two reference probes have been added and four replaced.
B2	Once reference probe has been replaced.
B1	Three reference probes have been replaced and one removed. QDX2 fragments have been added.
A2	Four reference probes have been replaced and four extra control fragments at 88-96-100-105 nt have been included.
A1	First release.

Implemented changes in the product description

Version B4-01– 19 April 2021 (04P)
- 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 *SERPINC1* gene updated according to new version of the NM_ reference sequence.

Version 10 – 5 October 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New references added on page 1.
- Various minor textual changes on pages 1 and 2.

Version 09 (53)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200