Product Description
SALSA® MLPA® Probemix P243-B1 SERPING1-F12

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 7.

Catalogue numbers:
- **P243-025R:** SALSA MLPA Probemix P243 SERPING1-F12, 25 reactions.
- **P243-050R:** SALSA MLPA Probemix P243 SERPING1-F12, 50 reactions.
- **P243-100R:** SALSA MLPA Probemix P243 SERPING1-F12, 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 P243 SERPING1-F12 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SERPING1* and *F12* genes, which are associated with hereditary angioedema (HAE).

HAE is a rare autosomal dominant disorder characterized by episodic local subcutaneous and submucosal oedema, involving the upper respiratory and gastrointestinal tracts. There are three types of HAE: type I, II, and III, which can be distinguished by underlying genetic cause and protein levels in the blood. HAE types I and II are primarily caused by defects in the *SERPING1* gene, encoding the C1 esterase inhibitor protein (C1NH), which inhibits activated C1r and C1s of the first complement component and thus regulates complement activation. In type I HAE, representing 85% of patients, serum levels of C1NH drop to less than 35%. In contrast, type II HAE is caused by non-functionality of the *SERPING1* gene, leaving protein levels normal or even elevated. The *SERPING1* gene is not involved in type III HAE, leaving the function and concentration of C1NH unaltered. Although type III is not fully explained, at least part of these patients harbour activating mutations in the *F12* (factor 12) gene.

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 *SERPING1* and *F12* exon numbering used in this P243-B1 SERPING1-F12 product description is the exon numbering from the LRG_105 and LRG_145 sequences, respectively. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG 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 P243-B1 SERPING1-F12 contains 33 MLPA probes with amplification products between 168 and 500 nucleotides (nt). This includes eight probes targeting all exons of the *SERPING1* gene and 13 probes for the *F12* gene, targeting all exons with the exception of exon 3. In addition, eleven 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 hereditary angioedema. 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 *SERPING1* and *F12* genes are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P243 SERPING1-F12.

- 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.

HAE mutation database

http://hae.enzim.hu/. We strongly encourage users to deposit positive results in the C1 inhibitor gene mutation database, or check the http://grenada.lumc.nl/LSDB_list/lsdbs/SERPING1 website for available *SERING1* specific mutation databases. 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 *SERPING1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P243 SERPING1-F12

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*SERPING1*	*F12*
64-105	Control fragments – see table in probemix content section for more information			
168	Reference probe 08222-L24899	10q		
178	Reference probe 02169-L13410	4q		
190 ¬	**APLNR probe** 05727-L05166		**Upstream**	
196	**SERPING1 probe** 08192-L08920		**Exon 6**	
202	**SERPING1 probe** 08187-L08081		**Exon 1**	
209	Reference probe 08637-L29011	3q		
214	**SERPING1 probe** 08191-L08085		**Exon 5**	
220	**SERPING1 probe** 08188-L08082		**Exon 2**	
227	Reference probe 01732-L01318	14q		
238	**SERPING1 probe** 08193-L08087		**Exon 7**	
247	**SERPING1 probe** 08189-L08083		**Exon 3**	
255	Reference probe 09899-L10312	16p		
265	**SERPING1 probe** 08194-L08088		**Exon 8**	
274	**SERPING1 probe** 08190-L08921		**Exon 4**	
283 «	**F12 probe** 20730-L29220			**Exon 7**
292 «	**F12 probe** 20731-L28632			**Exon 1**
301	Reference probe 06749-L06353	8q		
309 «	**F12 probe** 20732-L28633			**Exon 4**
328 «	**F12 probe** 20733-L28634			**Exon 8**
346	**F12 probe** 20734-L28635			**Exon 12**
362	**F12 probe** 20735-L28636			**Exon 10**
372	Reference probe 05953-L28763	2p		
382 «	**F12 probe** 20736-L28637			**Exon 2**
391	Reference probe 14984-L16720	6q		
400	**F12 probe** 20737-L28638			**Exon 13**
409	Reference probe 17462-L21218	12p		
419 «	**F12 probe** 20738-L28639			**Exon 9**
436	**F12 probe** 20739-L28640			**Exon 11**
448 «	**F12 probe** 20740-L28957			**Exon 6**
463	Reference probe 13538-L14998	19p		
474	**F12 probe** 20741-L28642			**Exon 14**
490 «	**F12 probe** 20742-L28643			**Exon 5**
500	Reference probe 14894-L27890	15q		

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

¬ 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.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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. P243-B1 probes arranged according to chromosomal location

Table 2a. *SERPING1*

Length (nt)	SALSA MLPA probe	*SERPING1* exon[a]	Ligation site NM_000062.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
190 ¬	05727-L05166	*APLNR* gene		CCAGTGCCTTCT-TCAGAATATCTG	364.1 kb
		start codon	*61-63 (Exon 2)*		
202	08187-L08081	Exon 1	16-17	TGCACTGGAGCT-GCCTGGTGACCA	0.8 kb
220	08188-L08082	Exon 2	129 nt after exon 2	TTGGGATCATTG-AGTGTGATCCTT	1.5 kb
247	08189-L08083	Exon 3	138-139	AATCCAAATGCT-ACCAGCTCCAGC	2.2 kb
274	08190-L08921	Exon 4	707-708	CTTCACGACCAA-AGGTGTCACCTC	3.9 kb
214	08191-L08085	Exon 5	813-814	CCCAGAGTCCTA-AGCAACAACAGT	0.3 kb
196	08192-L08920	Exon 6	953-952, reverse	TTGTCTTCCACT-TGGCTAGAGGGA	5.4 kb
238	08193-L08087	Exon 7	1122-1123	CACAATCTGAGT-TTGGTGATCCTG	2.5 kb
265	08194-L08088	Exon 8	88 nt before exon 8	TGGGACTCAGGA-TGAACCCAGAGA	
		stop codon	*1561-1563 (Exon 8)*		

Table 2b. *F12*

Length (nt)	SALSA MLPA probe	*F12* exon[a]	Ligation site NM_000505.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*38-40 (Exon 1)*		
292 «	20731-L28632	Exon 1	12-13	ACTCCTGGATAG-GCAGCTGGACCA	0.5 kb
382 «	20736-L28637	Exon 2	116-117	GGGAAGCCCCCA-AGGAGCATAAGT	3.3 kb
	No probe	Exon 3			
309 «	20732-L28633	Exon 4	12 nt before exon 4, reverse	CTGTAGAAAGAG-ACAAGGCTTCCC	0.5 kb
490 «	20742-L28643	Exon 5	423-422, reverse	CTTTCTGGCAGT-GGTTTCCAGTGA	0.2 kb
448 «	20740-L28957	Exon 6	531-532	CCAGTGCAAGGG-TCCTGATGCCCA	0.2 kb
283 «	20730-L29220	Exon 7	611-610, reverse	GTGGCCCTCCAC-CTCTAGGCAGCG	0.3 kb
328 «	20733-L28634	Exon 8	765-766	GCCGTGGGCCTC-GGAGGCCACCTA	0.2 kb
419 «	20738-L28639	Exon 9	895-896	GACCGGCTGAGC-TGGGAGTACTGC	0.3 kb
362	20735-L28636	Exon 10	1120-1121	AGCTGCGGGCAG-CGGCTCCGCAAG	0.5 kb
436	20739-L28640	Exon 11	1405-1406	TTCTCGCCCGTC-AGCTACCAGCAC	0.2 kb
346	20734-L28635	Exon 12	1498-1499	CCGGTGTGCCTG-CCAAGCGGCGCC	0.7 kb
400	20737-L28638	Exon 13	1651-1652	CCGGACGTGCAC-GGATCCTCCATC	0.4 kb
474	20741-L28642	Exon 14	1951-1952	GGGGCATGGAAG-GCAAGATTGTGT	
		stop codon	*1883-1885 (Exon 14)*		

[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.

¬ 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.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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 P243 SERPING1-F12

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- Ebo DG et al. (2018). Hereditary angioedema in 2 sisters due to paternal gonadal mosaicism. *J Allergy Clin Immunol Pract*, 6(1), 277-79.
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- Gökmen NM et al. (2019). Deletions in SERPING1 lead to lower C1 inhibitor function: lower C1 inhibitor function can predict disease severity. *Int Arch Allergy Immunol*, 178(1), 50-59.
- Grivčeva-Panovska V et al. (2018). Hereditary angioedema due to C1-inhibitor deficiency in Macedonia: clinical characteristics, novel SERPING1 mutations and genetic factors modifying the clinical phenotype. *Ann Med*, 50(3), 269-76.
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- Nabilou S et al. (2020). Genetic Study of Hereditary Angioedema Type I and Type II (First Report from Iranian Patients: Describing Three New Mutations). *Immunol Invest*, 1-12.
- Nicolicht P et al. (2019). Gene mapping strategy for Alu elements rearrangements: Detection of new large deletions in the SERPING1 gene causing hereditary angioedema in Brazilian families. *Gene*, 685, 179-185.
- Obtulowicz K et al. (2020). Genetic variants of SERPING1 gene in Polish patients with hereditary angioedema due to C1 inhibitor deficiency. *Cent Eur J Immunol*, 45(3), 301.
- Pedrosa M et al. (2016). Complement Study Versus CINH Gene Testing for the Diagnosis of Type I Hereditary Angioedema in Children. *J Clin Immunol*. 36:16-8.
- Ponard D et al. (2020). SERPING1 mutation update: Mutation spectrum and C1 Inhibitor phenotypes. *Hum mutat*, 41(1), 38-57.

P243 product history	
Version	*Modification*
B1	Probes for the *F12* gene have been included. Most reference probes have been replaced and new reference probes have been added.
A3	Three reference probes have been replaced and the control fragments have been adjusted (QDX2).
A2	One reference probe has been replaced and two extra reference probes, as well as four small control fragments at 88-96-100-105 nt, have been included.
A1	First release.

Implemented changes in the product description

Version B1-02 – 24 February 2022 (04P)
- Product description rewritten and adapted to a new template.
- Ligation sites of the probes targeting *SERPING1* and *F12* genes updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Warning added for salt sensitivity of probes detecting *F12* exons 1-9

Version B1-01 – 28 September 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2).

- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 10 – 7 September 2017 (55)

- Warning added in Table 1, 280 nt probe 20730-L29220, 292 nt probe 20731-L28632, 310 nt probe 20732-L28633, and 419 nt probe 20738-L28639.

Version 09 – 24 August 2016 (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).
- Included information about type III HAE and the *F12* gene at page 1.
- Name of the probemix is changed.
- New references added on page 2.
- Manufacturer's address adjusted.

Version 08 (53)

- 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).

Version 07 (48)

- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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