Product Description
SALSA® MLPA® Probemix P218-C2 LPL

To be used with the MLPA General Protocol.

Version C2
For complete product history see page 7.

Catalogue numbers:
- **P218-025R:** SALSA MLPA Probemix P218 LPL, 25 reactions.
- **P218-050R:** SALSA MLPA Probemix P218 LPL, 50 reactions.
- **P218-100R:** SALSA MLPA Probemix P218 LPL, 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 P218 LPL is a **research use only (RUO)** assay for the detection of deletions or duplications in the *LPL* gene, which is associated with lipoprotein lipase deficiency.

The *LPL* gene encodes lipoprotein lipase, which is expressed in heart, muscle, and adipose tissue. Lipoprotein lipase functions as a homodimer and has the dual function of triglyceride hydrolase and ligand/bridging factor for receptor-mediated lipoprotein uptake. Certain mutations can cause lipoprotein lipase deficiency, which is characterised by increase fatty acid levels, pancreatitis, hepatosplenomegaly and eruptive xanthomas, among others. Other mutations however, can result in conditions where the enzyme is overactive resulting in low fat levels.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1308/.

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 *LPL* exon numbering used in this P218-C2 LPL product description is the exon numbering from the LRG_1298 sequence. 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 P218-C2 LPL contains 25 MLPA probes with amplification products between 130 and 337 nucleotides (nt). This includes This includes 12 probes for the *LPL* gene, one probe for each of the ten exons and three probes for exon 1. Furthermore, two probes are included that target the upstream area of the *LPL* gene and two probes targeting the downstream area. 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.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 lipoprotein lipase 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 *LPL* gene are small (point) mutations, none of which will not be detected by using SALSA MLPA Probemix P218 LPL.

- 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.

***LPL* mutation database**

https://databases.lovd.nl/shared/genes/LPL. 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 SNVs and unusual results (e.g., a duplication of *LPL* exons 5 and 7 but not exon 6) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P218-C2 LPL

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*LPL*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L21056	5q	
143	**LPL probe** 17906-L22211		**Exon 1**
154 ¬	INTS10 probe 17907-L22212		Upstream
161	**LPL probe** 11721-L22597		**Exon 7**
172	Reference probe 00808-L00326	18q	
178	**LPL probe** 17900-L07550		**Exon 1**
184	**LPL probe** 06896-L22191		**Exon 8**
190	Reference probe 12422-L13423	14q	
202	Reference probe 04542-L03931	2q	
211	**LPL probe** 06894-L22192		**Exon 6**
219	**LPL probe** 06892-L22598		**Exon 4**
229	Reference probe 08679-L09778	13q	
233 ¬	INTS10 probe 17908-L22213		Upstream
244	Reference probe 11436-L12165	1q	
250	**LPL probe** 18211-L22195		**Exon 3**
256	Reference probe 14758-L16455	9q	
265	**LPL probe** 18210-L22194		**Exon 9**
274	**LPL probe** 06893-L24046		**Exon 5**
282	**LPL probe** 18212-L23563		**Exon 10**
292 ¬	SLC18A1 probe 17909-L22214		Downstream
300	**LPL probe** 06890-L22197		**Exon 2**
310	Reference probe 11344-L12069	12p	
319	**LPL probe** 17910-L22215		**Exon 1**
329 ¬	SLC18A1 probe 17911-L22216		Downstream
337	Reference probe 03264-L02701	3q	

[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.

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. *LPL* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*LPL* exon[a]	Ligation site NM_000237.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
154 ¬	17907-L22212	*INTS10* Exon 15		TTGCTTCAGCAA-GAGTGGCCACGG	7.5 kb
233 ¬	17908-L22213	*INTS10* Exon 17		AGGCATCACCAA-AGGCGTGAAGGA	87.4 kb
		start codon	*189-191 (Exon 1)*		
319	17910-L22215	Exon 1	143 nt before exon 1	TTCTCGTTGGCA-GGGTTGATCCTC	0.1 kb
143	17906-L22211	Exon 1	2 nt before exon 1 reverse	CGAGTCTGACAC-TGTTTTCACGCC	0.1 kb
178	17900-L07550	Exon 1	75-76	CCATCCCCTTTA-AAGGGCGACTTG	8.9 kb
300	06890-L22197	Exon 2	347-348	GCTGAGGACACT-TGCCACCTCATT	3.6 kb
250	18211-L22195	Exon 3	508-509	ACCAGACTCCAA-TGTCATTGTGGT	1.5 kb
219	06892-L22598	Exon 4	667-668	GGGATACAGCCT-TGGAGCCCATGC	0.9 kb
274	06893-L24046	Exon 5	914-915	TTTCAGCCAGGA-TGTAACATTGGA	1.7 kb
211	06894-L22192	Exon 6	1084-1085	AGCCTTTGAGAA-AGGGCTCTGCTT	3.3 kb
161	11721-L22597	Exon 7	3 nt before exon 7	TTTCCTCCCCAA-CAGTCTTCCATT	1.7 kb
184	06896-L22191	Exon 8	1416-1417	AGCTCAAATGGA-AGAGTGATTCAT	1.2 kb
265	18210-L22194	Exon 9	1534-1535	TTCTAGGGAGAA-AGTGTCTCATTT	3.6 kb
282	18212-L23563	Exon 10	2017-2018	GGACTGAGGCCT-TCTCAAACTTTA	179.9 kb
		stop codon	*1614-1616 (Exon 9)*		
329 ¬	17911-L22216	*SLC18A1* Exon 17		GTCCCTGATACT-CTTCTCACCTGT	37.5 kb
292 ¬	17909-L22214	*SLC18A1* Exon 1		GTCTTGCTAACA-GCTGCCAATACC	

[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.

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 P218 LPL

- Pugni L et al. (2014). Severe Hypertriglyceridemia in a Newborn with Monogenic Lipoprotein Lipase Deficiency: An Unconventional Therapeutic Approach with Exchange Transfusion. *JIMD Rep*. 13:59-64.
- Rabacchi C et al. (2015). Spectrum of mutations of the LPL gene identified in Italy in patients with severe hypertriglyceridemia. *Atherosclerosis*, 241(1), 79-86.
- Wright WT et al. (2008). Genetic screening of the LPL gene in hypertriglyceridaemic patients. *Atherosclerosis*, 199 (1), 187-192.

P218 product history	
Version	*Modification*
C2	One reference probe has been replaced.
C1	*LPL* probe for exon 1 has been replaced and three new *LPL* probes have been added. Four flanking probes have been added.
B1	All reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version C2-02 – 02 March 2022 (04P) - Product description rewritten and adapted to a new template. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. *Version C2-01 — 05 November 2019 (02P)* - Product description rewritten and adapted to a new template. *Version 09 (55) – 27 October 2015* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new pictures included). *Version 08 (54) – 15 July 2015* - Figure based on the use of old MLPA buffer (replaced in December 2012) removed. *Version 07 (49)* - Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new pictures included). - Various minor textual changes on page 1. - Various minor layout changes. - Electropherogram pictures included using the new MLPA buffer (introduced in December 2012).

More information: www.mrcholland.com; www.mrcholland.eu	
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