Instructions for Use

SALSA® MLPA® Probemix P062 LDLR

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P062 LDLR product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P062 LDLR**
Version	E1
Catalogue numbers	P062-025R (25 reactions) P062-050R (50 reactions) P062-100R (100 reactions)
Basic UDI-DI	872021148P0625S
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version D2, nine target probes for *LDLR* have been replaced, one target probe has been added (exon 18). The length of one target probe has been changed (no change in sequence detected). Five reference probes have been replaced. The length of two reference probes has been changed, however, no change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P062 LDLR is an in vitro diagnostic (IVD)[1] or research use only (RUO) semiquantitative manual assay[2] for the detection of deletions or duplications in the *LDLR* gene in genomic DNA isolated from human peripheral whole blood specimens. P062 LDLR is intended to confirm a potential cause for and clinical diagnosis of familial hypercholesterolaemia and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P062 LDLR should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *LDLR* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in combination with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types	
Test Sample	• Provided by user
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of familial hypercholesterolaemia.
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination
Positive Control Samples (Preferably)	• Provided by user.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*LDLR* gene)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used.

Cut-off values for copy number determination were verified with SALSA MLPA Probemix P062 LDLR in 39 samples from healthy individuals with normal copy number and 11 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested. For almost all measurements (999/1000), the expected final ratios (FRs) for the corresponding copy number were found in all samples tested. An ambiguous FRs was obtained for only one measurement in one positive sample, which would at most lead to delayed results.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance.

These lower and higher limits of detection were verified using SALSA MLPA Probemix P062 LDLR on three samples with normal copy number and expected results were obtained using both the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>SNPs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using SALSA MLPA Probemix P062 LDLR was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on two samples with known CNVs and one normal sample. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Expected FR for 174/180 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 180/180 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 178/180 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 179/180 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 170/180 measurements</td></tr>
</table>
* Results are summarised for 20 LDLR probes across all three samples tested.

NaCl did not interfere with copy number determination, while an effect on the FRs was observed for a low number of probes with $FeCl_3$ and heparin. The deviating ratios produced in these cases were ambiguous. This would only lead to delayed results. An effect on the FRs was observed for a low number of probes with EDTA. Haemoglobin had the largest effect on the FRs, as the wrong copy number was determined in several cases. DNA extraction methods from blood remove hemoglobin. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores.

To minimise variability across samples, 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.</td></tr>
<tr><td>Cross-reactions</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, two previously genotyped samples were tested using SALSA MLPA Probemix P062 LDLR and found to have the expected results in 40/40 measurements, leading to a trueness of >99% . Assay precision was tested by repeatedly testing three samples with known copy number status using SALSA MLPA Probemix P062 LDLR over multiple days, and by multiple operators. Results showed a correct call in 899/900 data points, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td>Approximately 50% of FH cases are caused by pathogenic variants in the LDLR gene. It is estimated that 10-15% of these pathogenic variants are attributed to large rearrangements (deletions and duplications)

*(Based on a 2005-2024 literature review)</td></tr>
</table>

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
19p13.2	*SMARCA4*		29.3 kb	142	02488-L13996	¬
19p13.2	*LDLR*	Upstream	0.3 kb	472	23262-L32894	Ø *
19p13.2	*LDLR*	Exon 1	10.8 kb	148	23261-L32893	+ *
19p13.2	*LDLR*	Exon 2	2.5 kb	160	02310-L01801	
19p13.2	*LDLR*	Exon 3	2.6 kb	184	23263-L32895	*
19p13.2	*LDLR*	Exon 4	1.3 kb	212	02314-L01805	
19p13.2	*LDLR*	Exon 5	0.8 kb	238	23264-L32896	*
19p13.2	*LDLR*	Exon 6	3.3 kb	292	23265-L32897	*
19p13.2	*LDLR*	Exon 7	0.9 kb	265	19521-L26015	
19p13.2	*LDLR*	Exon 8	1.8 kb	454	23266-L32898	*
19p13.2	*LDLR*	Exon 9	0.3 kb	364	02324-L26888	
19p13.2	*LDLR*	Exon 10	2.4 kb	390	19640-L26021	
19p13.2	*LDLR*	Exon 11	0.8 kb	328	19526-L26020	
19p13.2	*LDLR*	Exon 12	3.3 kb	193	02313-L01804	
19p13.2	*LDLR*	Exon 13	0.4 kb	400	23267-L32899	*
19p13.2	*LDLR*	Exon 14	2.5 kb	220	19333-L32957	+ ¥
19p13.2	*LDLR*	Exon 15	0.5 kb	247	19520-L26014	
19p13.2	*LDLR*	Intron 15 (Exon 15)	4.4 kb	427	10781-L11396	Ø
19p13.2	*LDLR*	Exon 16	1.6 kb	337	23268-L32900	*
19p13.2	*LDLR*	Exon 17	1.7 kb	318	23269-L32901	*
19p13.2	*LDLR*	Exon 18	0.4 kb	226	23270-L32958	*
19p13.2	*LDLR*	Exon 18		166	19517-L26011	
1p	Reference			154	14813-L16521	
2q	Reference			481	15318-L17117	*
3p	Reference			310	15380-L17211	
3q	Reference			202	05292-L21125	*
4q	Reference			283	14477-L16197	
6p	Reference			373	10718-L11300	*
7p	Reference			232	04360-L32960	¥
9q	Reference			256	18649-L29820	*
12q	Reference			415	18255-L22981	
15q	Reference			136	17174-L20399	*
17q	Reference			445	16634-L19164	
20q	Reference			463	16287-L32961	¥

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *LDLR* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com.
Annotation of one probe with a target at the edge of the coding region, is altered. The exon numbering from the product description of the previous product version is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe changes

*	New probes.
¥	Probes changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

¬	This probe is a flanking probe, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.
Ø	These probes target sequences outside of the known coding region. Copy number alterations of only (one of) these probes are of unknown clinical significance.
+	The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-

probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description
Version E1-01 – 14 January 2025 (03S) - Product description adapted to a new product version. - Intended purpose updated, specifying assay is manual. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites (affects probe: 10781-L11396). - Probemix is now IVDR-certified.