Product Description SALSA® MLPA® Probemix P484-A1 ALPL

To be used with the MLPA General Protocol.

Version A1.

Catalogue numbers:
- **P484-025R:** SALSA MLPA Probemix P484 ALPL, 25 reactions.
- **P484-050R:** SALSA MLPA Probemix P484 ALPL, 50 reactions.
- **P484-100R:** SALSA MLPA Probemix P484 ALPL, 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 P484 ALPL is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ALPL* gene, which is associated with hypophosphatasia (HPP).

HPP is a rare inherited metabolic disorder that is primarily characterised by reduced bone and dental mineralization. Severity and penetrance vary greatly: from stillbirth due to profound skeletal hypomineralization to adult-onset dental problems or arthropathies without bone disease. Severe forms are autosomal recessive with an estimate prevalence of 1:100,000-300,000; while less severe forms may be autosomal recessive or dominant and the prevalence may reach 1:6,000. Alleles associated with less severe dominant forms can have incomplete penetrance (Millan and Whyte 2016, Mornet 2017, Mornet 2018). Known pathogenic *ALPL* mutations cause loss of function of the tissue non-specific alkaline phosphatase (TNSALP) protein. Approximately 2-3% of pathogenic *ALPL* variants are deletions of one or more exons, but pathogenic whole gene deletions have not been found (Spentchian et al. 2006, Taillandier et al. 2017).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1150/

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 *ALPL* exon numbering used in this P484-A1 ALPL product description is the exon numbering from the RefSeq transcript NM_000478.6, which is identical to NG_008940.1 sequence. 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 P484-A1 ALPL contains 25 MLPA probes with amplification products between 130 and 310 nucleotides (nt). This includes 15 probes for the *ALPL* gene one probe for each of the 12 exons, two additional probes closely up- and downstream of exon 1, and a second probe for exon 12. In addition, ten 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 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 HPP. 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 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.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
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 *ALPL* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P484 ALPL.
- 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.

***ALPL* mutation database:** https://databases.lovd.nl/shared/genes/ALPL. 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 *ALPL* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P484-A1 ALPL

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	ALPL
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	**ALPL probe** 22045-L31005		**Exon 3**
142	Reference probe 19948-L27009	10q25	
148	**ALPL probe** 22046-L31269		**Exon 5**
154	Reference probe 10694-L11276	6p12	
160	**ALPL probe** 22047-L31007		**Exon 11**
166	**ALPL probe** 22048-L31008		**Exon 2**
173	Reference probe 19185-L27754	3q23	
177	**ALPL probe** 22049-L31009		**Exon 9**
184	**ALPL probe** 22050-L31010		**Exon 6**
190	Reference probe 12422-L13423	14q24	
196	**ALPL probe** 22051-L31011		**Exon 10**
202 « ๑	**ALPL probe** 22221-L31322		**Upstream**
211	Reference probe 15521-L17376	16q13	
218 ๑	**ALPL probe** 22053-L31013		**Exon 12**
229	**ALPL probe** 22054-L31014		**Exon 7**
238	Reference probe 20756-L28658	1q24	
247 « ๑	**ALPL probe** 22055-L31015		**Exon 1**
256	**ALPL probe** 22056-L31016		**Exon 12**
265	Reference probe 14759-L16456	11q23	
274	**ALPL probe** 22057-L31017		**Exon 8**
283	Reference probe 19759-L26542	9q34	
292	**ALPL probe** 22058-L31018		**Exon 4**
301 « Ø ๑	**ALPL probe** 22220-L31321		**Intron 1**
310	Reference probe 09065-L09234	19p13	

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

« 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.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
๑ The significance of copy number changes detected by this probe is not clear as it targets a sequence upstream of the *ALPL* gene or non-coding part of the gene.

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

Length (nt)	SALSA MLPA probe	ALPL exon[a]	Ligation site NM_00478.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
202 « ๑	22221-L31322	Upstream	626 nt before exon 1, reverse	GAGACGTAAAGG-GCCCGCAGGTGT	0.8 kb
247 « ๑	22055-L31015	Exon 1	82-83	GGACCCTCGCCA-GTGCTCTGCGCA	1.0 kb
301 « Ø ๑	22220-L31321	Intron 1	981 nt after exon 1	GCCTCAGTGTAG-ATACTGCAACTG	43.6 kb
		Start codon	*200-202 (Exon 2)*		
166	22048-L31008	Exon 2	216-217	TTCACCATTCTT-AGTACTGGCCAT	6.6 kb
136	22045-L31005	Exon 3	294-295	CTGGCGAGACCA-AGCGCAAGAGAC	0.4 kb
292	22058-L31018	Exon 4	382-383	CCCACTGCAGGG-ATGGGTGTCTCC	2.1 kb
148	22046-L31269	Exon 5	588-589	CACCGTGGGGGT-AAGCGCAGCCAC	1.0 kb
184	22050-L31010	Exon 6	836-837	TGCATAACATCA-GGGACATTGACG	3.9 kb
229	22054-L31014	Exon 7	895-896	AAGAATAAAACT-GATGTGGAGTAT	2.2 kb
274	22057-L31017	Exon 8	1033-1034	CTCCTGACCCTT-GACCCCCACAAT	3.3 kb
177	22049-L31009	Exon 9	1097-1096, reverse	GTTGTTCCTGTT-CAGCTCGTACTG	2.1 kb
196	22051-L31011	Exon 10	1228-1229	CACCATGAAGGA-AAAGCCAAGCAG	0.9 kb
160	22047-L31007	Exon 11	1492-1493	CGAGAGAATGTC-TCCATGGTGGAC	0.7 kb
256	22056-L31016	Exon 12	1518-1517, reverse	GCGCCTGGTAGT-TGTTGTGAGCTG	0.9 kb
		Stop codon	*1772-1774 (Exon 12)*		
218 ๑	22053-L31013	Exon 12	2373-2374	GGGAAGAGCCAC-CTGGCAGGGCTC	

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.

« 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.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
๑ The significance of copy number changes detected by this probe is not clear as it targets a sequence upstream of the *ALPL* gene or non-coding part of the gene.

References

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P484 Product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-02 — 06 August 2020 (02P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *ALPL* gene updated according to new version of the NM_ reference sequence.

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