Product Description
SALSA® MLPA® Probemix P387-A4 NPHP1

To be used with the MLPA General Protocol.

Version A4
As compared to version A3, one reference probe has been replaced. For complete product history see page 7.

Catalogue numbers:
- **P387-025R:** SALSA MLPA Probemix P387 NPHP1, 25 reactions.
- **P387-050R:** SALSA MLPA Probemix P387 NPHP1, 50 reactions.
- **P387-100R:** SALSA MLPA Probemix P387 NPHP1, 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 P387 NPHP1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *NPHP1* gene, which is associated with Senior-Loken syndrome type 1.

Familial juvenile nephronophthisis is characterized by a kidney disorder involving both tubules and glomeruli. Defects in the *NPHP1* gene on chromosome 2q13 is one of the causes of juvenile nephronophthisis. This protein appears to function in the control of cell division, as well as in cell-cell and cell-matrix adhesion signaling, likely as part of a multifunctional complex localized in actin- and microtubule-based structures.

Defects in this gene are also associated with Senior-Loken syndrome type 1, referred to as juvenile nephronophthisis with Leber amaurosis, which is characterized by kidney and eye disease. Furthermore, defects in the *NPHP1* gene are associated with Joubert syndrome type 4, characterized by cerebellar ataxia, oculomotor apraxia, psychomotor delay and neonatal breathing abnormalities, sometimes including retinal dystrophy and renal disease. Multiple transcript variants encoding different isoforms have been found for this gene.

The *NPHP1* gene (20 exons), spans ~82.7 kb of genomic DNA and is located on chromosome 2q13, 110.1 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK368475/.

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 *NPHP1* exon numbering used in this P387-A4 NPHP1 product description is the exon numbering from the exon numbering from the NG_008287.1 sequence. The exon numbering of the NM_000272.5 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 P387-A4 NPHP1 contains 30 MLPA probes with amplification products between 178 and 400 nucleotides (nt). This P387-A4 includes one probe for each exon of the *NPHP1* 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 Senior-Loken syndrome type 1. 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 *NPHP1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P387-A4 NPHP1.
- 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.

***NPHP1* mutation databases**

https://databases.lovd.nl/shared/genes/*NPHP1*. 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 *NPHP1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P387-A4 NPHP1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*NPHP1*
64-105	Control fragments – see table in probemix content section for more information		
178	Reference probe 13047-L14230	15q	
184	Reference probe 13764-L02494	4p	
190	**NPHP1** probe 16189-L18443		**Exon 5**
196	**NPHP1** probe 16190-L18444		**Exon 18**
202	Reference probe 10636-L13834	8q	
208	**NPHP1** probe 16191-L18445		**Exon 11**
214	**NPHP1** probe 16192-L20110		**Exon 3**
220	**NPHP1** probe 16193-L18447		**Exon 16**
226	Reference probe 07083-L06712	11p	
232	Reference probe 02334-L13114	12q	
238	Reference probe 12923-L14074	9q	
244 Ж	**NPHP1** probe 16194-SP0333-L18448		**Exon 2**
256	**NPHP1** probe 16196-L18450		**Exon 7**
265	Reference probe 11438-L12168	1q	
274	**NPHP1** probe 16197-L18451		**Exon 20**
283	**NPHP1** probe 16198-L18452		**Exon 4**
292	**NPHP1** probe 16207-L18461		**Exon 8**
297	**NPHP1** probe 16864-L19956		**Exon 1**
304	Reference probe 16436-L18889	18q	
310	**NPHP1** probe 16200-L20111		**Exon 10**
319	**NPHP1** probe 16201-L28219		**Exon 19**
326	**NPHP1** probe 16202-L18456		**Exon 6**
337	**NPHP1** probe 16869-L19662		**Exon 15**
344	**NPHP1** probe 16203-L18457		**Exon 17**
355	**NPHP1** probe 16204-L18458		**Exon 12**
364	**NPHP1** probe 16205-L18459		**Exon 9**
377 Ж	**NPHP1** probe 16982-SP0334-L18462		**Exon 14**
384	**NPHP1** probe 16206-L18460		**Exon 13**
391	Reference probe 14984-L16720	6q	
400 *	Reference probe 17588-L30994	7q	

[a] See section Exon numbering on page 2 for more information.

* New in version A4.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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. P387-A4 probes arranged according to chromosomal location

Tabel 2. *NPHP1* gene

Length (nt)	SALSA MLPA probe	*NPHP1* exon[a]	Ligation site NM_000272.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*46-48 (exon 1)*		
297	16864-L19956	Exon 1	9 nt before exon 1	CACCTTCTGGCG-CCGCCGGTTGGT	3.7 kb
244 Ж	16194-SP0333-L18448	Exon 2	103 nt and 145 nt after exon 2	CAAATGGAAGAA-42 nt spanning oligo-ACCTGATGGTTG	21.6 kb
214	16192-L20110	Exon 3	223-224	TAGATGAAAATA-AAAATGCTCTTC	1.2 kb
283	16198-L18452	Exon 4	330-331	CTTACCCAACAA-CTGCAGGGCCTT	8.6 kb
190	16189-L18443	Exon 5	512-513	AACCGGTGAAGA-ATACATCGCTGT	1.4 kb
326	16202-L18456	Exon 6	631-632	CTAAGGATGCCA-AAGGAAATGAAG	3.4 kb
256	16196-L18450	Exon 7	728-729	TGAAGAAGATGT-AGAGGCGGTGGA	0.5 kb
292	16207-L18461	Exon 8	890-891	TAGGATGGAGAC-TGTGGAAGACAC	1.5 kb
364	16205-L18459	Exon 9	1025-1026	GATGGGAGCTAT-TCCTGCAGGGTT	1.5 kb
310	16200-L20111	Exon 10	1124-1125	CATGCCTTCACA-ACTGGCCTTCAG	1.5 kb
208	16191-L18445	Exon 11	1269-1270	AGCAGACATGTA-CGCCTCTGTCTA	9.9 kb
355	16204-L18458	Exon 12	1340-1341	ATGGCAACCTAA-AAAGCCCAAAAC	2.2 kb
384	16206-L18460	Exon 13	1416-1417	GATTGCTTTATC-AGGTCTAATTCT	1.2 kb
377 Ж	16982-SP0334-L18462	Exon 14	1510-1511; 1543-1544	GAGAGTTAAGCT-33 nt spanning oligo-GTGGAGTTCCTA	2.2 kb
337	16869-L19662	Exon 15	8 nt before exon 15	TGTATGTTATTC-TTTTATAGAACT	1.0 kb
220	16193-L18447	Exon 16	1680-1681	ATGACAATGAGA-AGGCAGCCTCAA	11.9 kb
344	16203-L18457	Exon 17	1829-1830	TGTGCTCCTGAA-AGACAGGATGAG	2.5 kb
196	16190-L18444	Exon 18	1921-1922	ATGTGATGGATG-CTCTCAGGGTGG	3.5 kb
319	16201-L28219	Exon 19	1951-1952	CTGGAAAAGAAA-GCACATTAAAAA	1.8 kb
274	16197-L18451	Exon 20	2189-2190	TCATGAACCTTT-TGACCTTTCAGA	
		Stop Codon	*2245-2247 (exon 20)*		

[a] See section Exon numbering on page 2 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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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 P387-A4 NPHP1

- Abdelwahed, M et al. (2021) Copy-number variation of the NPHP1 gene in patients with juvenile Nephronophthisis. *Acta Clinica Belgica*, 76(1): 16-24.
- Haghighi A et al. (2016). Identification of an NPHP1 deletion causing adult form of nephronophthisis. *Irish Journal of Medical Science (1971-)* 185, 589-595.

- Jávorszky E et al. (2016). QMPSF is sensitive and specific in the detection of NPHP1 heterozygous deletions. *Clinical Chemistry and Laboratory Medicine* 55(6): 809-816.
- Obeidova, L et al. (2020) Results of targeted next-generation sequencing in children with cystic kidney diseases often change the clinical diagnosis. *PloS one*, 15(6): e0235071.

P387 product history	
Version	*Modification*
A4	One reference probe has been replaced.
A3	Three reference probes have been replaced.
A2	Two new reference probes are added.
A1	First release.

Implemented changes in the product description
Version A4-01– 16 December 2020 (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 *NPHP1* gene updated according to new versions of the NM_ reference sequences. Version 05 – 23 June 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). - New references added on page 1. Version 04 – 19 June 2015 (54) - Product description adapted to a new version (lot number added, small changes in Table 1 and Table 2, new picture included). - Minor textual change on page 1.

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