Product Description
SALSA® MLPA® Probemix P307-B4 SEPT9

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, five reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P307-025R:** SALSA MLPA Probemix P307 SEPT9, 25 reactions.
- **P307-050R:** SALSA MLPA Probemix P307 SEPT9, 50 reactions.
- **P307-100R:** SALSA MLPA Probemix P307 SEPT9, 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 P307 SEPT9 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SEPT9* gene, which is associated with Hereditary Neuralgic Amyopathy (HNA).

HNA is an autosomal dominant disorder that affects the brachial plexus and may first appear in childhood. It is characterized by episodes of sudden onset pain in arms and shoulders as well as weakness, followed by total or partial paralysis of the affected area. These episodes are often triggered by an infection, an immunization, childbirth, or overworking the arms and shoulders.

HNA has been associated with mutations in the *SEPT9* gene encoding Septin 9, a member of the Septin family. Septins are a group of evolutionarily conserved genes encoding proteins with various functions, including membrane transport, apoptosis, cell polarity, cell cycle regulation, cytokinesis, and oncogenesis. Septin 9 is ubiquitously expressed and it is believed that it is involved in cytokinesis and tumorigenesis.

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 *SEPT* exon numbering used in this P307-B4 SEPT9 product description is the exon numbering from the LRG_370 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 P307-B4 SEPT9 contains 29 MLPA probes with amplification products between 148 and 401 nucleotides (nt). This includes 19 probes for the *SEPT9* gene. Several of these probes target exons that are only present in certain transcript variants (see Table 2). 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 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 Neuralgic Amyopathy. 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 *SEPT9* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P307 SEPT9.
- 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.

***SEPT9* mutation database**

https://databases.lovd.nl/shared/genes/SEPT9. 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 *SEPT9* exons 7 and 9 but not exon 8) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P307-B4 SEPT9

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SEPT9*
64-105	Control fragments – see table in probemix content section for more information		
148	Reference probe 13478-L14940	1q	
152 *	Reference probe 14199-L25033	2q	
160	**SEPT9 probe** 20635-L28349		**Upstream**
172	**SEPT9 probe** 09649-L09807		**Exon 3**
183	**SEPT9 probe** 09645-L09803		**Exon 2**
190	**SEPT9 probe** 09654-L09812		**Exon 8**
196 *	Reference probe 10723-L11305	6p	
211	**SEPT9 probe** 09648-L09806		**Intron 2**
220 «	**SEPT9 probe** 10806-L09796		**Upstream**
229	**SEPT9 probe** 09641-L09799		**Exon 1**
238	**SEPT9 probe** 09650-L09808		**Exon 4**
247	Reference probe 10808-L11455	4q	
256	**SEPT9 probe** 09646-L09804		**Intron 2**
263	**SEPT9 probe** 20636-L28350		**Exon 7**
274	Reference probe 08887-L08849	12q	
283	**SEPT9 probe** 09657-L09815		**Exon 11**
292 «	**SEPT9 probe** 20634-L28348		**Upstream**
301 *	Reference probe 17637-L12634	3q	
310	**SEPT9 probe** 09647-L09805		**Intron 2**
319	**SEPT9 probe** 09655-L09813		**Exon 9**
328	Reference probe 19756-L26539	9q	
337	**SEPT9 probe** 09651-L09809		**Exon 5**
344	**SEPT9 probe** 09643-L09801		**Intron 1**
355 *	Reference probe 18692-L17111	2q	
363	**SEPT9 probe** 09642-L09800		**Exon 1**
373	**SEPT9 probe** 09656-L09814		**Exon 10**
382	Reference probe 16885-L19718	16q	
391	**SEPT9 probe** 09644-L09802		**Intron 1**
401 *	Reference probe 14865-L16589	14q	

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

* New in version B4.
« 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. *SEPT9* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SEPT9* exon[a]	Ligation site NM_006640.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*810-812 (Exon 1)*		
220 «	10806-L09796	Upstream	787 nt before exon 1 (NM_001113491.2)	TCGTGGAGACTT-TAAGGATATCCA	7.4 kb
292 «	20634-L28348	Upstream	72-73 (NM_001113492.2)	GTCTGGGTGAGA-GGAACCCTGGAT	19.0 kb
160	20635-L28349	Upstream	89 nt before exon 3 (NM_001113492.2)	CAGCAGGAGCAA-AGGCGAGGACAT	12.8 kb
229	09641-L09799	Exon 1	296-297	CTGTGAGTTGGT-TTCCAAGAGTCT	0.3 kb
363	09642-L09800	Exon 1	585-586	GACAATGCTACT-TCAGTTTGGAGC	53.4 kb
344	09643-L09801	Intron 1	210-211 (NM_001113493.2)	TCATTTCGGACT-TCGAAGGTGGGT	2.5 kb
391	09644-L09802	Intron 1	6-7 (NM_001113494.1)	GGAGAAGTCAGT-ATGGAGGAGGCG	26.2 kb
183	09645-L09803	Exon 2	1091-1090, reverse	GACGAGATGTCA-ATGGACAGCTCA	48.4 kb
256	09646-L09804	Intron 2	3-4 (NM_001113496.2)	AGCTGGATCAGA-TCTGAATCCAGA	24.6 kb
310	09647-L09805	Intron 2	119-120 (NM_001113495.1)	ATCTGAAGGACT-TTGCAGGCACCC	0.3 kb
211	09648-L09806	Intron 2	402-401 reverse (NM_001113495.1)	TGCCTCGTAACA-ATGCACACTGGA	6.7 kb
172	09649-L09807	Exon 3	1656-1657	TCGAGTTCAACA-TCATGGTGGTCG	5.2 kb
238	09650-L09808	Exon 4	1779-1780	AGACCATCGAGA-TCAAGTCCATCA	0.7 kb
337	09651-L09809	Exon 5	1809-1810	ATATTGAGGAGA-AAGGCGTCCGGA	2.5 kb
	No Probe	Exon 6			
263	20636-L28350	Exon 7	2058-2059	AACGCCTGAGCA-AGGTGGTCAACA	1.9 kb
190	09654-L09812	Exon 8	2187-2188	CCCAGAAGGAAT-TTGATGAGGACT	0.4 kb
319	09655-L09813	Exon 9	2288-2289	GTCAACGGCAAG-AGGATCCTTGGG	4.3 kb
373	09656-L09814	Exon 10	2348-2349	ACCACACACTGT-GAGTTTGCCTAC	1.2 kb
283	09657-L09815	Exon 11	2424-2425	GCAGCATCCACT-TCGAGGCGTACC	
		stop codon	*2514-2516 (Exon 11)*		

[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.

« 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.

Multiple alternatively spliced *SEPT9* transcript variants encoding different isoforms have been described:

- NM_006640.5 represents transcript variant 3 and is a reference standard in the RefSeqGene project.
- NM_001113491.2 represents transcript variant 1 which is the longest isoform.
- NM_001113492.2 represents transcript variant 5 which has an alternative 5' exon.
- NM_001113493.2 represents transcript variant 2 which lacks two 5' exons but has an alternative 5' exon as compared to variant 1.
- NM_001113494.1 represents transcript variant 6 which lacks two 5' exons but has an alternative 5' exon, which results in a downstream AUG start codon, as compared to variant 1.
- NM_001113495.1 represents transcript variant 4 which lacks three 5' exons but has an alternative 5' exon as compared to variant 1.
- NM_001113496.2 represents transcript variant 7 which lacks three 5' exons but has an alternative 5' exon as compared to variant 1.

Related SALSA MLPA probemixes

P033 CMT1 Contains probes for the *PMP22* gene (linked to HNPP).

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 publication using SALSA MLPA Probemix P307 SEPT9

- Neubauer, K et al. (2019). Hereditary neuralgic amyotrophy in childhood caused by duplication within the SEPT9 gene: A family study. *Cytoskeleton*, 76(1), 131-136.Publication 2

P307 product history	
Version	*Modification*
B4	Five reference probes have been replaced.
B3	One reference probe has been replaced.
B2	Two reference probes have been replaced. In addition, the control fragments have been adjusted (QDX2).
B1	One SEPT9 and one reference probe have been removed.
A1	First release.

Implemented changes in the product description

Version B4-01 – 22 July 2022 (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 *SEPT9* gene 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.

Version B3-01 – 18 December 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, new picture included).

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