Product Description SALSA® MLPA® Probemix P307-B3 SEPT9

To be used with the MLPA General Protocol.

Version B3. As compared to version B2, one reference probe has been replaced. For complete product history see page 6.

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, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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 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/

Probemix content: The SALSA MLPA Probemix P307-B3 SEPT9 contains 29 MLPA probes with amplification products between 148 and 409 nt. This includes 19 probes for the *SEPT9* gene. Several of these probes target exons that are only present in certain transcript variants. In addition, ten reference probes are included and detect ten different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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.mlpa.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 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 105 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 HNA. 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 Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample 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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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 or pseudo-autosomal chromosomes:

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 (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 are unlikely to have any relation to the condition tested for.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *SEPT9* gene are small (point) mutations, most of which will not 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. 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.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *SEPT9* exons 7 and 9 but not exon 8) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P307-B3 SEPT9

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	SEPT9
64-105	Control fragments – see table in probemix content section for more information		
148	Reference probe 13478-L14940	1q42	
154	Reference probe 08825-L08885	2p13	
160	**SEPT9 probe** 20635-L28349		**Upstream**
172	**SEPT9 probe** 09649-L09807		**Exon 3**
181	**SEPT9 probe** 09645-L09803		**Exon 2**
190	**SEPT9 probe** 09654-L09812		**Exon 8**
202	Reference probe 08546-L08547	3q24	
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	4q25	
256	**SEPT9 probe** 09646-L09804		**Intron 2**
263	**SEPT9 probe** 20636-L28350		**Exon 7**
274	Reference probe 08887-L08849	12q21	
283	**SEPT9 probe** 09657-L09815		**Exon 11**
292	**SEPT9 probe** 20634-L28348		**Upstream**
301	Reference probe 10615-L11166	15q15	
310	**SEPT9 probe** 09647-L09805		**Intron 2**
319	**SEPT9 probe** 09655-L09813		**Exon 9**
328	Reference probe 19756-L26539	9q34	
337	**SEPT9 probe** 09651-L09809		**Exon 5**
344	**SEPT9 probe** 09643-L09801		**Intron 1**
355	Reference probe 19167-L25691	4q31	
363	**SEPT9 probe** 09642-L09800		**Exon 1**
373	**SEPT9 probe** 09656-L09814		**Exon 10**
382 *	Reference probe 16885-L19718	16q22	
391	**SEPT9 probe** 09644-L09802		**Intron 1**
409	Reference probe 09615-L09910	20p12	

* New in version B3 (from lot B3-1018 onwards).

Note: The exon numbering used in this P307-B3 SEPT9 product description is the exon numbering from the RefSeq transcript NM_006640.4, which is identical to the LRG_370 sequence. The exon numbering and NM sequence used is from 12/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2. SEPT9 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	SEPT9 Exon	Ligation site NM_006640.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
220	10806-L09796	Upstream (1)	787 nt before exon 1 (NM_001113491.2)	TCGTGGAGACTT-TAAGGATATCCA	7.4 kb
292	20634-L28348	Upstream (2)	116-117 (NM_001113492.1)	GTCTGGGTGAGA-GGAACCCTGGAT	19.0 kb
160	20635-L28349	Upstream (3)	89 nt before exon 3 (NM_001113492.1)	CAGCAGGAGCAA-AGGCGAGGACAT	12.8 kb
		start codon	*813-815 (exon 1)*		
229	09641-L09799	Exon 1 (4)	299-300	CTGTGAGTTGGT-TTCCAAGAGTCT	0.3 kb
363	09642-L09800	Exon 1 (4)	588-589	GACAATGCTACT-TCAGTTTGGAGC	53.4 kb
344	09643-L09801	Intron 1 (5)	357-358 (NM_001113493.1)	TCATTTCGGACT-TCGAAGGTGGGT	2.5 kb
391	09644-L09802	Intron 1 (6)	6-7 (NM_001113494.1)	GGAGAAGTCAGT-ATGGAGGAGGCG	26.2 kb
181	09645-L09803	Exon 2 (7)	1094-1093 reverse	GACGAGATGTCA-ATGGACAGCTCA	48.4 kb

Length (nt)	SALSA MLPA probe	SEPT9 Exon	Ligation site NM_006640.4	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
256	09646-L09804	Intron 2 (8)	221-222 (NM_001113496.1)	AGCTGGATCAGA-TCTGAATCCAGA	24.6 kb
310	09647-L09805	Intron 2 (9)	119-120 (NM_001113495.1)	ATCTGAAGGACT-TTGCAGGCACCC	0.3 kb
211	09648-L09806	Intron 2 (9)	402-401 reverse (NM_001113495.1)	TGCCTCGTAACA-ATGCACACTGGA	6.7 kb
172	09649-L09807	Exon 3 (10)	1659-1660	TCGAGTTCAACA-TCATGGTGGTCG	5.2 kb
238	09650-L09808	Exon 4 (11)	1782-1783	AGACCATCGAGA-TCAAGTCCATCA	0.7 kb
337	09651-L09809	Exon 5 (12)	1812-1813	ATATTGAGGAGA-AAGGCGTCCGGA	2.5 kb
	No probe	*Exon 6 (13)*			
263	20636-L28350	Exon 7 (14)	2061-2062	AACGCCTGAGCA-AGGTGGTCAACA	1.9 kb
190	09654-L09812	Exon 8 (15)	2190-2191	CCCAGAAGGAAT-TTGATGAGGACT	0.4 kb
319	09655-L09813	Exon 9 (16)	2291-2292	GTCAACGGCAAG-AGGATCCTTGGG	4.3 kb
373	09656-L09814	Exon 10 (17)	2351-2352	ACCACACACTGT-GAGTTTGCCTAC	1.2 kb
283	09657-L09815	Exon 11 (18)	2427-2428	GCAGCATCCACT-TCGAGGCGTACC	
		stop codon	*2517-2519 (exon 11)*		

Note: The exon numbering used in this P307-B3 SEPT9 product description is the exon numbering from the RefSeq transcript NM_006640.4, which is identical to the LRG_370 sequence. The exon numbering and NM sequence used is from 12/2018, but can be changed (e.g. by NCBI) after the release of the product description. The exon numbering used in previous versions of this product description can be found between brackets! Please notify us of any mistakes: info@mlpa.com.

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

NM_006640.4 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_001113492.1 represents transcript variant 5 which has an alternative 5' exon.
NM_001113493.1 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_001113496.1 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.

P307 Product history	
Version	*Modification*
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 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.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200