Product Description SALSA® MLPA® Probemix P010-B1 POLG

To be used with the MLPA General Protocol.

Version B1. As compared to version A4, one target probe has been removed and two probe lengths have been adjusted. For complete product history see page 7.

Catalogue numbers:
- **P010-025R:** SALSA MLPA Probemix P010 POLG, 25 reactions.
- **P010-050R:** SALSA MLPA Probemix P010 POLG, 50 reactions.
- **P010-100R:** SALSA MLPA Probemix P010 POLG, 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 P010 POLG is a **research use only (RUO)** assay for the detection of deletions or duplications in the genes *POLG*, *POLG2*, *TWNK* and *SLC25A4*, which are associated with autosomal disorders of mitochondrial DNA (mtDNA) maintenance.

The genes *POLG* (15q26.1), *POLG2* (17q23.3), *TWNK* (10q24.31) and *SLC25A4* (4q35.1) are involved in the replication of mtDNA, a process crucial to ensure cellular energy production. Pathogenic variants of those genes therefore lead to mtDNA depletion and/or accumulation of mtDNA deletions as well as energy deficiency within affected cells. Insufficient cellular energy production subsequently results in organ dysfunction. The most frequently affected organs and tissues are the brain, liver, skeletal muscle, peripheral nerves and gastrointestinal tract. MtDNA maintenance disorders are therefore classified into groups depending on the predominantly affected organ and range from early-onset pediatric encephalopathic syndromes to late-onset myopathy with chronic progressive external ophthalmoplegia.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK487393

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 POLG exon numbering used in this P010-B1 POLG product description is the exon numbering from the RefSeq transcript NM_002693.2, which is identical to the LRG_765 sequence. The POLG2 exon numbering is the exon numbering from the RefSeq transcript NM_007215.4, which is identical to the NG_013029.1 sequence, and the TWNK exon numbering from the RefSeq transcript NM_021830.5 is identical to the NG_012624.1 sequence. The SLC25A4 exon numbering is the exon numbering from the RefSeq transcript NM_001151.3, which is identical to the LRG_441 sequence. The exon numbering and NM_ sequence used have been retrieved on 09/2020. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P010-B1 POLG contains 44 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 23 probes for the *POLG* gene, three probes for the *POLG2* gene, five probes for the *TWNK* gene, and four probes for the *SLC25A4* gene. In addition, nine 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.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 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 mtDNA maintenance disorders. It is recommended to use samples of the same sex to facilitate interpretation. 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 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 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 genes *POLG*, *POLG2*, *TWNK* and *SLC25A4* are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P010 POLG.
- 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.

Mutation databases:
POLG: https://databases.lovd.nl/shared/genes/POLG
POLG2: https://databases.lovd.nl/shared/genes/POLG2
TWNK: https://databases.lovd.nl/shared/genes/C10orf2
SLC25A4: https://databases.lovd.nl/shared/genes/SLC25A4

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 *POLG* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P010-B1 POLG

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*POLG*	Other
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q31		
136 ±	**POLG probe** 11208-L11891		**Exon 6**	
142	**POLG probe** 11216-L11899		**Exon 14**	
148 ±	**POLG probe** 11205-L11888		**Exon 3**	
154	Reference probe 10051-L10475	8q22		
160	**POLG probe** 11212-L11895		**Exon 10**	
166	**TWNK probe** 11194-L11877			***TWNK* exon 1**
172	**POLG probe** 11209-L11892		**Exon 7**	
178	Reference probe 08019-L07800	11q24		
184	**POLG probe** 13430-L14885		**Exon 13**	
190	**TWNK probe** 12408-L14695			***TWNK* exon 2**
196	**POLG probe** 11204-L11887		**Exon 2**	
209	**POLG probe** 11217-L11900		**Exon 15**	
214	**TWNK probe** 11197-L11880			***TWNK* exon 4**
220	**POLG probe** 11214-L14693		**Exon 12**	
227 ¥	Reference probe 12269-L32197	22q13		
232 ¥	**POLG probe** 11220-L32198		**Exon 18**	
238	**POLG probe** 11224-L11907		**Exon 22**	
245	**POLG probe** 11213-L14694		**Exon 11**	
252	**POLG probe** 11221-L11904		**Exon 19**	
272	**SLC25A4 probe** 11226-L11909			***SLC25A4* exon 1**
281	Reference probe 18919-L25190	6q25		
292	**POLG probe** 11203-L11886		**Exon 1**	
301	**POLG2 probe** 11202-L11885			***POLG2* exon 8**
310	**SLC25A4 probe** 11227-L11910			***SLC25A4* exon 2**
319	Reference probe 08048-L07829	5p15		
328	**POLG probe** 11218-L11901		**Exon 16**	
335	**SLC25A4 probe** 11228-L11911			***SLC25A4* exon 3**
346	**POLG probe** 12407-L11902		**Exon 17**	
355	Reference probe 05595-L05046	14q21		
364	**POLG probe** 11207-L11890		**Exon 5**	
373	**SLC25A4 probe** 11229-L11912			***SLC25A4* exon 4**
389	**POLG probe** 11211-L29702		**Exon 9**	
399	**POLG probe** 11222-L11905		**Exon 20**	
409 Δ «	**TWNK probe** 11196-L11879			***TWNK* exon 3**
418	**POLG probe** 11206-L11889		**Exon 4**	
427	Reference probe 17154-L20346	1p22		
436	**POLG probe** 11223-L11906		**Exon 21**	
445 «	**TWNK probe** 11198-L11881			***TWNK* exon 5**
454	**POLG probe** 11210-L11893		**Exon 8**	
472	**POLG2 probe** 11200-L11883			***POLG2* exon 2**
484	**POLG probe** 11225-L11908		**Exon 23**	
494	**POLG2 probe** 11201-L11884			***POLG2* exon 5**
500	Reference probe 17001-L22947	20q11		

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

¥ Changed in version B1. Minor alteration, no change in sequence detected.
± SNP rs145289229 and the *POLG* c.752C>T (T251I) mutation could influence the signal of the exon 6 probe (136 nt) and the exon 3 probe (148 nt), respectively. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.

Table 2. P010-B1 probes arranged according to chromosomal location

Table 2a. *SLC25A4* (4q53.1)

Length (nt)	SALSA MLPA probe	*SLC25A4* exon[a]	Ligation site NM_001151.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*108-110 (Exon 1)*		
272	11226-L11909	Exon 1	214-215	CAAACTGCTGCT-GCAGGTGAGGAC	1.7 kb
310	11227-L11910	Exon 2	601-602	CATCAAGATCTT-CAAGTCTGATGG	0.7 kb
335	11228-L11911	Exon 3	738-739	ACGTGCACATTT-TTGTGAGCTGGA	1.1 kb
373	11229-L11912	Exon 4	893-894	AGGAAGATTGCA-AAAGACGAAGGA	
		stop codon	*1002-1004 (Exon 4)*		

Table 2b. *TWNK* (10q24.31)

Length (nt)	SALSA MLPA probe	*TWNK* exon[a]	Ligation site NM_021830.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*669-671 (Exon 1)*		
166	11194-L11877	Exon 1	471-472	GTGGACGCGAAA-GGGTCGTGTAGA	1.7 kb
190	12408-L14695	Exon 2	2013-2014	AGATCAGCAATG-TGAGACTAGCCC	0.8 kb
409 Δ «	11196-L11879	Exon 3	2230-2231	CCTGCAGTTCAT-GATGGGTCACGA	0.4 kb
214	11197-L11880	Exon 4	2313-2314	TTGCAACAGACA-ATAACTGCCATG	2.4 kb
445 «	11198-L11881	Exon 5	2513-2514	GGAGATGTAGGT-GTCTTCCCGCTT	
		stop codon	*2721-2723 (Exon 5)*		

Table 2c. *POLG* (15q26.1)

Length (nt)	SALSA MLPA probe	*POLG* exon[a]	Ligation site NM_002693.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*312-314 (Exon 2)*		
292	11203-L11886	Exon 1	26 nt before exon 1	TAGCTGGGTGCA-GACGGGAAGTTG	1.0 kb
196	11204-L11887	Exon 2	193-194	ACCCATTTCACT-GACAGGAGAGCA	3.7 kb
148 ±	11205-L11888	Exon 3	1071-1072	CTACTGGTGCCA-GCAGCCCCACCC	1.1 kb
418	11206-L11889	Exon 4	1194-1195	TCCTGGACACCA-TGAGCATGCACA	0.3 kb
364	11207-L11890	Exon 5	1387-1388	TCTGGCAGAGGT-GCACAGACTTTA	0.2 kb
136 ±	11208-L11891	Exon 6	1497-1498	TGATGCAGTACT-GTGCCCAGGACG	1.2 kb
172	11209-L11892	Exon 7	1623-1624	ACCTGCCTGTCA-ACCAGAACTGGG	0.2 kb
454	11210-L11893	Exon 8	1750-1751	CTGTAGGTACAA-AGAAGACCCCTG	0.3 kb
389	11211-L29702	Exon 9	1927-1928	GGAGGAGGAGTT-TCAACAAGATGT	1.1 kb
160	11212-L11895	Exon 10	2139-2140	CCTGGGATGGCT-TCCCTCTGCACT	1.4 kb
245	11213-L14694	Exon 11	2346-2345, reverse	AGTGAGCAGGAA-CTCCTCCGCCAG	0.3 kb
220	11214-L14693	Exon 12	2455-2456	AGTGCCAGGTCA-ACCCCTAGCTCT	0.3 kb
184	13430-L14885	Exon 13	39 nt before exon 13	CTGAATGCAGGT-GCTGGAGCAGGG	0.7 kb
142	11216-L11899	Exon 14	2593-2594	TAATAGCTGTAA-TGTGGGAAGCCC	0.9 kb
209	11217-L11900	Exon 15	2790-2791	GTGCTGTGATCA-GGTATGGTCTGC	0.2 kb
328	11218-L11901	Exon 16	2860-2861	TGCCGGCACCAT-CACTCGCCGGGC	0.6 kb
346	12407-L11902	Exon 17	2941-2942	GTTGAAAGCCAT-GGTGCAGGCCCC	0.3 kb
232	11220-L32198	Exon 18	3122-3123	AAGACAGCCACT-ACTGTGGGCATC	1.6 kb
252	11221-L11904	Exon 19	3336-3337	TGAGGGAGTTGA-ACCTCCCAGTGG	0.3 kb
399	11222-L11905	Exon 20	3521-3522	TCTGACATACCA-CGTACCCCGGTG	0.3 kb
436	11223-L11906	Exon 21	3618-3619	GGGTGGTACAGA-GCTCTGCTGTTG	1.2 kb
238	11224-L11907	Exon 22	3822-3823	AGCTGGGTCTGA-ATGACTTGCCCC	0.9 kb
484	11225-L11908	Exon 23	4161-4162	TCAGTAGCAGGA-CCTGCCAAGAAG	
		stop codon	*4029-4031 (Exon 23)*		

Table 2d. *POLG2* (17q23.3)

Length (nt)	SALSA MLPA probe	*POLG2* exon[a]	Ligation site NM_007215.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*87-89 (Exon 1)*		
472	11200-L11883	Exon 2	703-704	GAGGCTACCTTA-TGGCCTTGCTCA	7.2 kb
494	11201-L11884	Exon 5	1161-1162	AGCTGACAGAGA-ACTCCTTTACAA	7.8 kb
301	11202-L11885	Exon 8	1435-1436	TACTTTGGAGAA-TGGATTAATACA	
		stop codon	*1542-1544 (Exon 8)*		

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.

± SNP rs145289229 and the *POLG* c.752C>T (T251I) mutation could influence the signal of the exon 6 probe (136 nt) and the exon 3 probe (148 nt), respectively. In case of apparent deletions, it is recommended to sequence the region targeted by these probes.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
« 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.

Related SALSA MLPA probemixes

P089 TK2 Contains probes for the genes *TK2*, *DGUOK*, *MPV17*, *RRM2B*, *SUCLA2* and *SUCLG1*, which are associated with mitochondrial DNA depletion syndromes.

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 P010 POLG

- Naess K et al. (2012). Complete deletion of a POLG1 allele in a patient with Alpers syndrome. *JIMD Rep.* 4:67-73.
- Piekutowska-Abramczuk D et al. (2018). The frequency of mitochondrial polymerase gamma related disorders in a large Polish population cohort. *Mitochondrion*. 47:179-87.

P010 Product history	
Version	*Modification*
B1	One target probe has been removed and two probe lengths have been adjusted.
A4	One reference probe has been replaced and one reference probe has been removed. The probe lengths of one target probe has been adjusted.
A3	Two reference probes have been replaced and the control fragments have been adjusted (QDX2).
A2	Five reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version B1-01 — 16 November 2020 (02P) - 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 genes *POLG*, *POLG2*, *TWNK* and *SLC25A4* updated according to new version of the NM_ reference sequence.

Version 07 (55) - 28 February 2017

- 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).
- Gene name of C10orf2 is adjusted to TWNK which is the HGNC Approved Gene Symbol.

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