Product Description SALSA® MLPA® Probemix P309-B2 MTM1

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, five reference probes have been replaced and one probe has been adjusted in length. For complete product history see page 7.

Catalogue numbers:
- **P309-025R:** SALSA MLPA Probemix P309 MTM1, 25 reactions.
- **P309-050R:** SALSA MLPA Probemix P309 MTM1, 50 reactions.
- **P309-100R:** SALSA MLPA Probemix P309 MTM1, 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 P309 MTM1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MTM1* and *MTMR1* genes, which are associated with X-linked myotubular myopathy.

X-linked myotubular myopathy, also known as myotubular myopathy, is characterised by progressive muscle weakness (myopathy) and decreased muscle tone (hypotonia) that can range from mild to severe. Defects in the *MTM1* gene on chromosome Xq28 are the main cause of X-linked myotubular myopathy. The protein encoded by this gene is myotubularin, a dual-specificity phosphoinositide-3-phosphatase. The *MTMR1* gene is located at very short distance from the *MTM1* gene. The two genes share a similar genomic structure, suggesting that they originate from an intrachromosomal gene duplication. Deletions including both (or part of) *MTM1* and *MTMR1* have been described. However, deletion of the *MTMR1* gene has little clinical significance during early postnatal life (Zanoteli et al. 2005).

The *MTM1* gene (15 exons) spans ~105 kb of genomic DNA and is located on chromosome Xq28, ~150 Mb from the p-telomere. The *MTMR1* gene (16 exons) spans ~72 kb of genomic DNA and is also located on chromosome Xq28, 20 kb telomeric to the *MTM1* gene.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1432/.

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 *MTM1* exon numbering used in this P309-B2 MTM1 product description is the exon numbering from the LRG_839 sequence. The *MTMR1* exon numbering is the exon numbering from the RefSeq transcript NM_003828.4, which is identical to the NG_012551.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 07/2020. 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 P309-B2 MTM1 contains 35 MLPA probes with amplification products between 130 and 418 nucleotides (nt). This includes 14 probes for the *MTM1* gene, one probe for each exon with the exception of exon 12, and seven probes for the *MTMR1* gene. Furthermore, this probemix also contains three flanking probes located elsewhere on Xq28. In addition, 11 reference probes are included that detect locations on the X-chromosome. 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 of the same sex 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 X-linked myotubular myopathy. 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 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:

Copy Number status: Male samples	**Dosage quotient**
Normal	0.80 < DQ < 1.20
Deletion	DQ = 0
Duplication	1.65 < DQ < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	**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 *MTM1* and *MTMR1* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P309 MTM1.
- 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.

MTM1 **and** ***MTMR1*** **mutation databases:** https://databases.lovd.nl/shared/genes/MTM1 and https://databases.lovd.nl/shared/genes/MTMR1. 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 *MTM1* exons 5 and 7 but not exon 6) to MRC Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P309-B2 MTM1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	MTM1	MTMR1	Other
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 13499-L02104	Xp11			
136	**MTM1** probe 12647-L11339		**Exon 3**		
142 ¥	**MTM1** probe 21830-L32247		**Exon 11**		
148 ¬	DKC1 probe 08626-L08642				Xq28
154	**MTMR1** probe 10760-L11356			**Exon 12**	
160	**MTM1** probe 10744-L11340		**Exon 4**		
165	Reference probe 07099-L06679	Xp22			
172	**MTM1** probe 12327-L13330		**Exon 5**		
178	**MTM1** probe 10748-L12835		**Exon 8**		
184 *	Reference probe 12594-L13678	Xq12			
196	**MTMR1** probe 10757-L11353			**Exon 3**	
202	Reference probe 13742-L15229	Xp22			
208	**MTM1** probe 10741-L12837		**Exon 2**		
214	**MTM1** probe 10750-L26023		**Exon 10**		
220	Reference probe 05864-L05264	Xq22			
228	**MTMR1** probe 10759-L11355			**Exon 10**	
238 *	Reference probe 19561-L26140	Xq26			
247	**MTMR1** probe 10758-L11354			**Exon 5**	
256	**MTM1** probe 12328-L13331		**Exon 7**		
265 *	Reference probe 19389-L25796	Xp11			
274	**MTM1** probe 12650-L13723		**Exon 6**		
283	**MTMR1** probe 10761-L12953			**Exon 13**	
292 *	Reference probe 03122-L02562	Xq21			
301	**MTM1** probe 12649-L13332		**Exon 13**		
310	**MTM1** probe 10755-L11351		**Exon 15**		
319 ¬	IDSP1 probe 05251-L04631				Xq28
328	**MTM1** probe 10749-L11345		**Exon 9**		
340	**MTM1** probe 12330-L25669		**Exon 1**		
351	Reference probe 01952-L01065	Xp21			
373 *	Reference probe 01282-L00965	Xq22			
387	**MTM1** probe 10754-L11350		**Exon 14**		
393 «	**MTMR1** probe 10756-L11352			**Exon 2**	
400 « ¬	FLNA probe 04608-L08387				Xq28
409	**MTMR1** probe 10762-L11358			**Exon 15**	
418	Reference probe 06473-L05999	Xp22			

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

* New in version B2.
¥ Changed in version B2. Minor alteration, no change in sequence detected.
« 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2. *MTM1*/*MTMR1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
319 ¬	05251-L04631	*IDSP1* gene		CTTGGAGGAAAT-GAGTGAAAAGAT	1070.9 kb
		MTM1 gene	**NM_000252.3**		
		start codon	*53-55 (Exon 2)*		
340	12330-L25669	Exon 1	529 nt after exon 1	GTTTCTGTGTCA-TACACGAGAGGT	23.4 kb
208	10741-L12837	Exon 2	51-52	TAGAGTTTCCAG-GATGGCTTCTGC	3.9 kb
136	12647-L11339	Exon 3	135-136	AGATGGAGTCAA-TCGAGATCTCAC	2.1 kb
160	10744-L11340	Exon 4	230-231	ATGGCCCCATTA-AGGGAAGAGTTT	16.0 kb
172	12327-L13330	Exon 5	323-324	TGGGTGTGATCT-CGAGAATTGAAA	4.5 kb
274	12650-L13723	Exon 6	448-449	AGCAGAAGAGAT-ATGTTTGAGATC	20.0 kb
256	12328-L13331	Exon 7	27 nt after exon 7	CTATTGTCTGGT-ATGTGATGAACC	2.3 kb
178	10748-L12835	Exon 8	707-708	GAGTTGCAACTT-TTAGGTCCCGAA	4.3 kb
328	10749-L11345	Exon 9	772-773	ACGGTCATTGTG-CGTTGCAGTCAG	4.0 kb
214	10750-L26023	Exon 10	958-959	GATGCATATCAT-AACGCCGAACTT	8.1 kb
142	21830-L32247	Exon 11	1158-1159	TTCAGGGAAGAG-TTCAGTGCTTGT	2.7 kb
	No probe	Exon 12			
301	12649-L13332	Exon 13	127 nt after exon 13	AGGAATTACATT-AAAGGTATGCAT	3.0 kb
387	10754-L11350	Exon 14	1668-1669	GAATTACTACAT-TAGATGGAACCC	7.9 kb
310	10755-L11351	Exon 15	1755-1756	CGACGAATACAT-AAAGCGGCTTGA	27.7 kb
		stop codon	*1862-1864 (Exon 15)*		
		MTMR1 gene	**NM_003828.4**		
		start codon	*136-138 (Exon 1)*		
393 «	10756-L11352	Exon 2	310-311	TTGAATGGTGTA-AACAGCTTATAG	19.5 kb
196	10757-L11353	Exon 3	450-451	CCAGGAGAATCA-ATTAAAGCCATT	9.1 kb
247	10758-L11354	Exon 5	629-630	AGCACAGAGCCA-TGGAGACAATTC	8.9 kb
228	10759-L11355	Exon 10	1219-1220	ATGAAAGTGAAA-GTGCTTACCCAA	7.8 kb
154	10760-L11356	Exon 12	1606-1607	ATGGTAATGACA-ACCATGCGGATG	6.5 kb
283	10761-L12953	Exon 13	1783-1784	AACAGCAGCGAT-TCAAAGAGGTGA	11.7 kb
409	10762-L11358	Exon 15	1985-1986	CATTCACCAGAA-TCTCAAGGAGCT	3560.0 kb
		stop codon	*2131-2133 (Exon 15)*		
400 « ¬	04608-L08387	*FLNA* gene		CACGCACACCAT-TACCTACATTCC	406.8 kb
148 ¬	08626-L08642	*DKC1* gene		CGGCTGCACAAT-GCTATTGAAGGG	

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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

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.
- Zanoteli E et al. (2005). Deletion of both MTM1 and MTMR1 genes in a boy with myotubular myopathy. *Am J Med Genet A*. 134:338-340.

Selected publications using SALSA MLPA Probemix P309 MTM1

- Biancalana V et al. (2017). Affected female carriers of MTM1 mutations display a wide spectrum of clinical and pathological involvement: delineating diagnostic clues. *Acta Neuropathol*. 134:889-904.
- Longo G et al. (2016). Mutation spectrum of the MTM1 gene in XLMTM patients: 10 years of experience in prenatal and postnatal diagnosis. *Clin Genet*. 89:93-8.
- Oliveira J et al. (2013). Expanding the MTM1 mutational spectrum: novel variants including the first multi-exonic duplication and development of a locus-specific database. *Eur J Hum Genet.* 21:540-549.
- Trump N et al. (2012). X-linked myotubular myopathy due to a complex rearrangement involving a duplication of MTM1 exon 10. *Neuromuscul Disord.* 22:384-388.

P309 Product history	
Version	*Modification*
B2	Five reference probes have been replaced and one probe has been adjusted in length.
B1	Two target specific probes and one flanking probe have been removed, three reference probes have been replaced. Furthermore the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version B2-01 — 21 July 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).
- Various minor textual or layout changes.
- Ligation sites of the probes targeting the *MTM1* and *MTMR1* genes updated according to new version of the NM_ reference sequence.

Version 07 – 20 January 2017 (55)
- Warning added in Table 1 and Table 2, 393 nt probe 10756-L11352 and 400 nt probe 04608-L08387.

Version 06 – 05 October 2016 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 2, new pictures included).
- Various textual changes.
- References added on page 2.
- Ligation sites of the probes targeting the *MTMR1* gene updated according to new version of the NM_reference sequence.

Version 05 (52)
- 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).

Version 04 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 03 (47)
- Various minor textual changes on page 1 and 2.
- Minor changes in the data analysis section on page 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Remark on RefSeqGene standard added below Table 2.
- Small corrections of chromosomal locations in Table 1.

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