Product Description
SALSA® MLPA® Probemix P143-C3 MFN2-MPZ

To be used with the MLPA General Protocol.

Version C3
As compared to version C2, five reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P143-025R:** SALSA MLPA Probemix P143 MFN2-MPZ, 25 reactions.
- **P143-050R:** SALSA MLPA Probemix P143 MFN2-MPZ, 50 reactions.
- **P143-100R:** SALSA MLPA Probemix P143 MFN2-MPZ, 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 P143 MFN2-MPZ is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MFN2* and *MPZ* genes, which are associated with Charcot-Marie-Tooth disease type 2A and type 1B, respectively.

Charcot-Marie-Tooth disease constitutes a clinically and genetically heterogeneous group of hereditary motor and sensory neuropathies, affecting approximately 1 in every 2500 individuals. On the basis of electrophysiological criteria, CMT is divided into 2 major types. Type 1, the demyelinating form, is characterised by a slow motor median nerve conduction velocity. Type 2, the axonal form, has normal or slightly reduced nerve conduction velocity.

Mitofusins, such as MFN2, mediate the fusion of mitochondria and thereby contribute to the dynamic balance between fusion and fission that determines mitochondrial morphology. Mutations in the *MFN2* gene have been detected in affected members of several families with Charcot-Marie-Tooth disease type 2A (CMT2A).

Myelin protein zero (MPZ) is the major structural protein of peripheral myelin. Mutations in the *MPZ* gene are associated with the autosomal dominant form of Charcot-Marie-Tooth disease type 1 (CMT1B) which is characterised by progressive slowing of nerve conduction and hypertrophy of Schwann cells. Mutations in *MPZ* can also produce the more severe polyneuropathies Dejerine-Sottas syndrome (DSS) and congenital hypomyelinating neuropathy (CHN), as well as several types of axonal CMT2.

The *MFN2* gene (19 exons) spans ~33 kb of genomic DNA and is located on 1p36.22, 12 Mb from the p-telomere. The *MPZ* gene (6 exons) spans ~5 kb of genomic DNA and is located on 1q23.3, ~160 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1358/ and https://www.ncbi.nlm.nih.gov/books/NBK1511/.

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 *MFN2* exon numbering used in this P143-C3 MFN2-MPZ product description is the exon numbering from the LRG_255 sequence. The *MPZ* exon numbering is the exon numbering from the LRG_256 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 P143-C3 MFN2-MPZ contains 38 MLPA probes with amplification products between 137 and 427 nucleotides (nt). This includes 20 probes for the *MFN2* gene (one probe for each exon and two probes for exon 3) and seven probes for the *MPZ* gene (one probe for each exon and two probes for exon 1). Two probes upstream of *MFN2*, targeting the *PLOD1* gene, are included. Please note that duplications of *PLOD1* exons 10-16 are found in the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA) (Giunta et al. 2005). 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.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 Charcot-Marie-Tooth disease. 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. Sample ID number NA00803 from the Coriell Institute has been tested with this P143-C3 probemix at MRC Holland and can be used as a positive control sample to detect a heterozygous deletion of the *MPZ* gene. 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 *MFN2* and *MPZ* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P143 MFN2-MPZ.
- 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.

***MFN2* and *MPZ* mutation databases**
https://databases.lovd.nl/shared/genes/MFN2 and https://databases.lovd.nl/shared/genes/MPZ. 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 *MFN2* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P143-C3 MFN2-MPZ

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*MFN2*	*MPZ*
64-105	Control fragments – see table in probemix content section for more information			
137 *	Reference probe 21914-L30711	4q		
142	Reference probe 06967-L06547	1q		
148	**MFN2 probe** 04886-L05353		**Exon 11**	
154	**MPZ probe** 04895-L04279			**Exon 1**
160	Reference probe 06669-L06242	10p		
166	**MFN2 probe** 04877-L04261		**Exon 2**	
172	**MFN2 probe** 04887-L04271		**Exon 12**	
178	**MPZ probe** 04896-L04280			**Exon 2**
184	**MFN2 probe** 05674-L29924		**Exon 3**	
190	**MFN2 probe** 06137-L05581		**Exon 5**	
196	**MFN2 probe** 04888-L04272		**Exon 13**	
202	**MPZ probe** 04897-L04281			**Exon 3**
208	Reference probe 06733-L16568	22q		
214	**MFN2 probe** 04879-L04263		**Exon 4**	
220	**MFN2 probe** 04889-L29925		**Exon 14**	
226	**MPZ probe** 06139-L29923			**Exon 1**
232	**MPZ probe** 04898-L08284			**Exon 4**
240	**MFN2 probe** 06138-L29700		**Exon 1**	
247 *	Reference probe 21928-L30731	15q		
256	**MFN2 probe** 04890-L05354		**Exon 15**	
265	**MPZ probe** 04899-L04283			**Exon 5**
274 *	Reference probe 18336-L23249	14q		
283	**MFN2 probe** 04881-L04265		**Exon 6**	
292	**MFN2 probe** 04891-L04275		**Exon 16**	
301	**MPZ probe** 04900-L04284			**Exon 6**
310 ¬	PLOD1 probe 04686-L04064		13 kb upstream of *MFN2*	
320	**MFN2 probe** 04882-L04266		**Exon 7**	
328	**MFN2 probe** 04892-L04276		**Exon 17**	
337 ¬	PLOD1 probe 04685-L04063		20 kb upstream of *MFN2*	
346	**MFN2 probe** 04883-L04267		**Exon 8**	
355	**MFN2 probe** 04893-L04277		**Exon 18**	
365 *	Reference probe 22419-L31604	19q		
373	**MFN2 probe** 04884-L04268		**Exon 9**	
382	**MFN2 probe** 04894-L04278		**Exon 19**	
390 *	Reference probe 20969-L12842	2q		
410	**MFN2 probe** 20882-L24212		**Exon 10**	
418	**MFN2 probe** 06136-L29926		**Exon 3**	
427	Reference probe 05561-L04993	7p		

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

* New in version C3.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the *PLOD1* probes are unlikely to be related to CMT. However, duplications of PLOD1 exons 10-16 are found in the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA) (Giunta et al. 2005).

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. P143-C3 probes arranged according to chromosomal location

Table 2a. *MFN2* gene

Length (nt)	SALSA MLPA probe	*MFN2* exon[a]	Ligation site NM_014874.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
337 ¬	04685-L04063	*PLOD1* gene Exon 10	NM_000302.4; 1091-1092	GCATGGCAGCGA-GTACCAGTCTGT	6.3 kb
310 ¬	04686-L04064	*PLOD1* gene Exon 16	NM_000302.4; 1750-1751	CCATCTTCACGG-AGGTGGCCTGTG	13.2 kb
		start codon	*191-193 (Exon 3)*		
240	06138-L29700	Exon 1	239 nt before exon 1	GAGTCCGAGCCT-CTGCGTCGTCCG	1.8 kb
166	04877-L04261	Exon 2	74-75	CAGTCAATCAAT-AGCCAACCTCAA	7.2 kb
184	05674-L29924	Exon 3	215-216	TCTCTCGATGCA-ACTCTATCGTCA	0.1 kb
418	06136-L29926	Exon 3	344-343 *reverse*	GGTGGCGCTCTC-CTGGATGTAGGC	3.3 kb
214	04879-L04263	Exon 4	430-431	GACGTCAAAGGT-TACCTATCCAAA	3.6 kb
190	06137-L05581	Exon 5	530-531	GCACCGTGATCA-ATGCCATGCTCT	1.2 kb
283	04881-L04265	Exon 6	750-751	GCCCAACTCTAA-GTGCCCACTTCT	1.4 kb
320	04882-L04266	Exon 7	820-821	ACAGAGCTGGAC-AGCTGGATTGAC	0.3 kb
346	04883-L04267	Exon 8	971-972	ACAACCGCTGGG-ATGCATCTGCCT	2.4 kb
373	04884-L04268	Exon 9	1105-1106	TTCTTTGTGTCT-GCTAAGGAGGTG	0.3 kb
410	20882-L24212	Exon 10	1182-1183	CGCAGAAGGCTT-TCAAGTGAGGAT	0.3 kb
148	04886-L05353	Exon 11	1309-1310	GAGGCGGTTCGA-CTCATCATGGAC	2.0 kb
172	04887-L04271	Exon 12	1435-1436	GCTCAAGACTAT-AAGCTGCGAATT	0.5 kb
196	04888-L04272	Exon 13	1531-1532	CTGGTGGACGAT-TACCAGATGGAC	0.3 kb
220	04889-L29925	Exon 14	1606-1607	CACATAGAGGAA-GGACTGGGTCGA	1.0 kb
256	04890-L05354	Exon 15	1852-1853	ATGCTGGTGAAT-AGGTTCCTGGGC	0.7 kb
292	04891-L04275	Exon 16	1972-1973	CAGGGCTCGCTC-ACCCAGGAGGAG	0.6 kb
328	04892-L04276	Exon 17	2222-2223	AGCTTGTCATCA-GCTACACTGGCT	2.5 kb
355	04893-L04277	Exon 18	2331-2332	GGAGCAGGAAAT-TGCCGCCATGAA	1.9 kb
382	04894-L04278	Exon 19	2432-2433	AGCTCAACATGT-TCACACACCAGT	
		stop codon	*2462-2464 (Exon 19)*		

Table 2b. *MPZ* gene

Length (nt)	SALSA MLPA probe	*MPZ* exon[a]	Ligation site NM_000530.8	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*64-66 (Exon 1)*		
226	06139-L29923	Exon 1	85 nt before exon 1	CTGCACATGCCA-GGCTGCAATTGG	0.2 kb
154	04895-L04279	Exon 1	88-89	CTCCCTCATCCA-GCCCCAGCCCTA	2.6 kb
178	04896-L04280	Exon 2	217-218	TGCACTGCTCCT-TCTGGTCCAGTG	0.5 kb
202	04897-L04281	Exon 3	386-387	CCCTCGCTGGAA-GGATGGCTCCAT	0.4 kb
232	04898-L08284	Exon 4	536-537	CGGGGTCGTTCT-GGGAGCTGTGAT	0.3 kb
265	04899-L04283	Exon 5	678-679	TTGCACAAGCCA-GGAAAGGACGCG	0.2 kb
301	04900-L04284	Exon 6	733-734	ATGCAATGCTGG-ACCACAGCAGAA	
		stop codon	*808-810 (Exon 6)*		

[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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the *PLOD1* probes are unlikely to be related to CMT. However, duplications of PLOD1 exons 10-16 are found in the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA) (Giunta et al. 2005).

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.

Related SALSA MLPA probemixes

- P033 CMT1: Charcot-Marie-Tooth disease (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) – genes included: *KIF1B,* 17p12 region (including *PMP22*).
- P405 CMT1: Charcot-Marie-Tooth disease (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) – genes included: *MPZ*, *GJB1*, 17p12 region (including *PMP22*).

References

- Giunta C et al. (2005). Mutation analysis of the PLOD1 gene: an efficient multistep approach to the molecular diagnosis of the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VIA). *Mol Genet Metab*. 86:269-276.
- 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 P143 MFN2-MPZ

- Baets J et al. Genetic spectrum of hereditary neuropathies with onset in the first year of life. *Brain*. 134:2664-2676.
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- Høyer H et al. (2015). Copy number variations in a population-based study of Charcot-Marie-Tooth disease. *BioMed Res Int*. 2015.
- Kotruchow K et al. (2015). Pathogenic mutations and sequence variants within mitofusin 2 gene in Polish patients with different hereditary motor-sensory neuropathies. *Acta Neurobiol Exp (Wars)*. 75:264-278.
- Østern R et al. (2013). Diagnostic laboratory testing for Charcot Marie Tooth disease (CMT): the spectrum of gene defects in Norwegian patients with CMT and its implications for future genetic test strategies. *BMC Med Genet*. 14:94.
- Polke JM et al. (2011). Recessive axonal Charcot-Marie-Tooth disease due to compound heterozygous mitofusin 2 mutations. *Neurology*. 77:168-73.
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P143 product history	
Version	*Modification*
C3	Five reference probes have been replaced.
C2	One reference probe has been removed and two reference probes have been replaced. In addition, several probe lengths have been adjusted.
C1	One *MFN2* exon 1 probe has been removed, and two reference probes have been replaced. Also, the control fragments have been adjusted (QDX2).
B1	Seven reference probes have been replaced and four extra control fragments at 88, 96, 100 and 105 nt have been included.

Implemented changes in the product description
Version C3-01– 09 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 *MFN2*, *MPZ* and *PLOD1* genes updated according to new versions of the NM_ reference sequences. - Removed P129 GJB1 from related SALSA MLPA probemixes, because this probemix was discontinued. *Version 09 – 25 April 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). - Various minor textual changes on pages 1 and 2. - New references added on page 1. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

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