Product Description SALSA® MLPA® Probemix P418-B2 MYH7

To be used with the MLPA General Protocol.

Version B2. Compared to version B1, one reference probe has been removed and one reference probe has been replaced. For complete product history see page 6.

Catalogue numbers:
- **P418-025R:** SALSA MLPA Probemix P418 MYH7, 25 reactions.
- **P418-050R:** SALSA MLPA Probemix P418 MYH7, 50 reactions.
- **P418-100R:** SALSA MLPA Probemix P418 MYH7, 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 P418 MYH7 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *MYH7* gene, which is associated with Hypertrophic Cardiomyopathy (HCM).

HCM is characterised by a cardiovascular disease with autosomal dominant inheritance. HCM is caused by defects in the genes coding for structural and/or regulatory proteins found in the sarcomere of cardiomyocytes. In ~20% of HCM patients, mutations in the *MYH7* gene on chromosome 14q11 are found. This gene encodes the beta heavy chain subunit of cardiac myosin.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1768/.

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 *MYH7* exon numbering used in this P418-B2 MYH7 product description is the exon numbering from the RefSeq transcript NM_000257.2, which is identical to the LRG_384 sequence. The exon numbering and NM_ sequence used have been retrieved on 04/2020. 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 P418-B2 MYH7 contains 46 MLPA probes with amplification products between 132 and 504 nucleotides (nt). This includes 37 probes for the *MYH7* gene, one probe for each exon of the gene, with the exception of exons 10, 31, 35, 36, and 37, and two probes for exons 9 and 40. 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 cardiomyopathy. 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 *MYH7* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P418 MYH7.
- 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.

***MYH7* mutation database:** https://databases.lovd.nl/shared/genes/MYH7. 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 *MYH7* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P418-B2 MYH7

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	MYH7
64-105	Control fragments – see table in probemix content section for more information		
132	Reference probe 00797-L21698	5q31	
137	Reference probe 13526-L14986	3q21	
148	**MYH7 probe** 17966-L22298		**Exon 22**
154	**MYH7 probe** 17967-L22299		**Exon 11**
166	**MYH7 probe** 17969-L22301		**Exon 17**
172	**MYH7 probe** 17970-L22302		**Exon 3**
178	Reference probe 10676-L20612	6p12	
184 Ж	**MYH7 probe** 17971-SP0584-L23607		**Exon 32**
190 Ж	**MYH7 probe** 17972-SP0585-L22304		**Exon 20**
194 Ж	**MYH7 probe** 17973-SP0586-L22305		**Exon 2**
202	**MYH7 probe** 17974-L22306		**Exon 39**
207	**MYH7 probe** 17975-L22307		**Exon 27**
220 Ж	**MYH7 probe** 17977-SP0587-L22309		**Exon 24**
226 *	Reference probe 17445-L21201	12p13	
232 Ж	**MYH7 probe** 17978-SP0588-L22310		**Exon 38**
238	**MYH7 probe** 17979-L22311		**Exon 12**
244	**MYH7 probe** 17980-L22882		**Exon 9**
251 Ж	**MYH7 probe** 17981-SP0589-L22963		**Exon 1**
258 Ж	**MYH7 probe** 17982-SP0590-L22314		**Exon 16**
267 Ж	**MYH7 probe** 17984-SP0591-L22316		**Exon 40**
274	**MYH7 probe** 17983-L22964		**Exon 40**
283	**MYH7 probe** 17985-L22965		**Exon 13**
292	Reference probe 08617-L22966	17q23	
300	**MYH7 probe** 17986-L22967		**Exon 28**
307	**MYH7 probe** 17987-L22968		**Exon 19**
313 Ж	**MYH7 probe** 17988-SP0592-L22969		**Exon 33**
322	**MYH7 probe** 17989-L22321		**Exon 4**
332 Ж	**MYH7 probe** 17990-SP0593-L22322		**Exon 18**
340	**MYH7 probe** 17991-L22323		**Exon 34**
359	**MYH7 probe** 17993-L22325		**Exon 5**
365 Ж	**MYH7 probe** 17995-SP0594-L22327		**Exon 8**
375 ¥	**MYH7 probe** 22723-L22326		**Exon 9**
382	Reference probe 13329-L14755	18q21	
396	**MYH7 probe** 17996-L22328		**Exon 29**
403	**MYH7 probe** 17997-L22329		**Exon 7**
411 Ж	**MYH7 probe** 17998-SP0595-L22330		**Exon 23**
418	Reference probe 10394-L22885	9q34	
427	**MYH7 probe** 17999-L22331		**Exon 25**
438 Ж	**MYH7 probe** 18000-SP0596-L22332		**Exon 6**
448 Ж	**MYH7 probe** 18001-SP0597-L22333		**Exon 30**
459	**MYH7 probe** 18002-L22334		**Exon 15**
467	**MYH7 probe** 18003-L22335		**Exon 21**
477 Ж	**MYH7 probe** 18004-SP0598-L22336		**Exon 14**
485 Ж	**MYH7 probe** 18005-SP0599-L22337		**Exon 26**
494	Reference probe 15203-L16978	3p12	
504	Reference probe 09870-L19465	2p15	

* New in version B2.

¥ Changed in version B2. Minor alteration, no change in sequence detected.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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

Table 2. MYH7 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	MYH7 exon[a]	Ligation site NM_000257.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*106-108 (Exon 3)*		
251 Ж	17981-SP0589-L22963	Exon 1	22-23; 12 nt after exon 1	CCTGCTCTGTGT-31nt spanning oligo-GCTGGAGGCTTT	1.4 kb
194 Ж	17973-SP0586-L22305	Exon 2	5 nt before exon 2; 64-65	CTCTTCCCTCTC-29nt spanning oligo-TTCCTCATCTGT	0.6 kb
172	17970-L22302	Exon 3	259-260	AGGCCAAGATCG-TGTCTCGAGAGG	0.4 kb
322 #	17989-L22321	Exon 4	334-335	AGGACCAGGTGA-TGCAGCAGAACC	0.4 kb
359	17993-L22325	Exon 5	3 nt before exon 5	CTTTTCTATCCC-CAGACCTACTCG	0.3 kb
438 Ж	18000-SP0596-L22332	Exon 6	3 nt before exon 6; 7 nt after exon 6	ACCCTCTCCCCA-27nt spanning oligo-TAGCTGCTACTG	0.6 kb
403 #	17997-L22329	Exon 7	660-661	GCAGGGAAGACA-GTCAACACCAAG	0.2 kb
365 Ж	17995-SP0594-L22327	Exon 8	1 nt before exon 8; 778-779	TTGGCCTCTGCA-35nt spanning oligo-CTGCTCTGGAGG	0.1 kb
244	17980-L22882	Exon 9	29 nt before exon 9	GCCTTCCCCCAA-CTCATCACCACT	0.1 kb
375	22723-L22326	Exon 9	32 nt after exon 9	AGGCTCAGCCTA-AGCTCACCCTTG	0.7 kb
154	17967-L22299	Exon 11	25 nt before exon 11	GGCTTGTGTCCC-ACCCTAACCATG	0.9 kb
238	17979-L22311	Exon 12	48 nt after exon 12, reverse	CTGAGCAGACAT-GGCCCTCCATGA	0.4 kb
283	17985-L22965	Exon 13	33 nt before exon 13	GCAGTCATCTCT-TTACCAACTTTG	0.3 kb
477 Ж	18004-SP0598-L22336	Exon 14	8 nt before exon 14; 1389-1390	GTCTCTCCTCCA-35nt spanning oligo-AAGGCAGTGTAT	0.6 kb
459	18002-L22334	Exon 15	8 nt after exon 15, reverse	GGTAAGGCCAAA-GAGGCACCTTCT	0.8 kb
258 Ж	17982-SP0590-L22314	Exon 16	1827-1828; 1878-1879	GGGAAGCCTGAA-51nt spanning oligo-GGCTGGCTGCAG	0.4 kb
166	17969-L22301	Exon 17	2057-2058	GTCAGCTCTGCA-CAGGGTGAGTGG	0.4 kb
332 Ж	17990-SP0593-L22322	Exon 18	2095-2096; 2124-2125	CCAACTTGCGCT-29nt spanning oligo-ATCCCTAATGAG	0.7 kb
307	17987-L22968	Exon 19	5 nt before exon 19	CTTCCTTCTTGC-CACAGGGGTGAT	0.4 kb
190 Ж	17972-SP0585-L22304	Exon 20	2366-2367; 4 nt after exon 20	CATTGATCACAA-29nt spanning oligo-GGAAAGGAGACT	0.2 kb
467	18003-L22335	Exon 21	12 nt before exon 21	GGCCAACACACA-CCTTGCCTGCAG	0.7 kb
148	17966-L22298	Exon 22	4 nt after exon 22	TGCAGGCGGTGA-GGCTCCTGGGCT	0.7 kb
411 Ж	17998-SP0595-L22330	Exon 23	2802-2803; 2837-2838	GACAACCTGGCA-35nt spanning oligo- CAAGATTCAGCT	0.6 kb
220 Ж	17977-SP0587-L22309	Exon 24	3177-3178; 6 nt after exon 24	ACTAAGGCCAAA-33nt spanning oligo-AGATTGAGAGTT	1.7 kb
427	17999-L22331	Exon 25	324 nt after exon 25	CATGGAGGAAGG-CTCCACATGAGA	0.9 kb
485 Ж	18005-SP0599-L22337	Exon 26	3410-3411; 3434-3435	GGCCCTCGGCAG-24nt spanning oligo-GCTTCAGGTGAG	0.7 kb
207	17975-L22307	Exon 27	63 nt before exon 27	GAGAGCCTTTTA-GAGCCGGGGGGA	0.7 kb
300	17986-L22967	Exon 28	3859-3858, reverse	CTGGTCTTCCAA-GGTCCGGCACAT	0.4 kb
396	17996-L22328	Exon 29	4039-4040	ACACCCAGCAGC-TGGAGGACCTCA	1.0 kb
448 Ж	18001-SP0597-L22333	Exon 30	intron-4274; 4245-4244, reverse	CTCAGAACTCAC-30nt spanning oligo-ATGGCGTCCGTC	0.9 kb
184 Ж #	17971-SP0584-L23607	Exon 32	4500-4499; 2 nt before exon 32, reverse	GACTCCAGCTCC-44nt spanning oligo-CCCGGGGACAAG	0.4 kb
313 Ж	17988-SP0592-L22969	Exon 33	4717-4718; 4746-4747	AGGCCGAGAAGA-29nt spanning oligo-GAGGTGTGTGTG	0.8 kb
340	17991-L22323	Exon 34	2 nt after exon 34, reverse	TCTGGGTGAGTA-CCTTCAACAAGC	2.0 kb
232 Ж	17978-SP0588-L22310	Exon 38	5724-5725; 5754-5755	AAGCTGCAGCTA-30nt spanning oligo-GAGGCGGTGAGT	0.3 kb
202	17974-L22306	Exon 39	5890-5891	GTGACATTGGCA-CGAAGGTGGGTC	0.9 kb
274	17983-L22964	Exon 40	38 nt before exon 40	CCCAATACCATC-TCTCCAAGGACT	0.1 kb
267 Ж	17984-SP0591-L22316	Exon 40	5954-5955; 5986-5987	GGAGGTGCCAGC-32nt spanning oligo-CTTGGGAGGAAG	
		stop codon	*5911-5913 (Exon 40)*		

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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.

Related SALSA MLPA probemixes

P100 MYBPC3	Contains probes for the *MYBPC3* gene involved in familial HCM.
P168 ARVC-PKP2	Contains probes for the *DSC2*, *DSP*, *JUP*, *PKP2*, *RYR2,* and *TGFB3* genes involved in Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVC).
P196 TNNT2-BAG3	Contains probes for the *TNNT2* and *BAG3* genes involved in HCM and Dilated Cardiomyopathy.

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.

P418 Product history	
Version	*Modification*
B2	One reference probe has been removed and one reference probe has been replaced.
B1	Three target probes have been removed.
A1	First release.

Implemented changes in the product description

Version B2-01 — 04 May 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 *MYH7* 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 06 – 18 May 2018 (55)
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 05 – 24 June 2016 (55)
- Product description adapted to a new version (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 04 – 14 December 2015 (55)
- Warning added in Table 1 and 2, 160 nt probe 17968-SP0583-L22880 and 214 nt probe 17976-L22308.
- Warning adjusted in Table 1 and 2, for 348 nt probe 17992-L23188.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

- Note II about the MM_57965.2 sequence deleted.
- Various minor textual changes on page 1 and 2.

Version 03 (53)
- Broader explanation about SNP rs61737004 added.
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 02 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 01 (48)
- Not applicable, new document.

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