Product Description SALSA® MLPA® Probemix P058-A4 IGHMBP2

To be used with the MLPA General Protocol.

Version A4. Compared to version A3, three reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P058-025R:** SALSA MLPA Probemix P058 IGHMBP2, 25 reactions.
- **P058-050R:** SALSA MLPA Probemix P058 IGHMBP2, 50 reactions.
- **P058-100R:** SALSA MLPA Probemix P058 IGHMBP2, 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 P058 IGHMBP2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *IGHMBP2* gene, which is associated with spinal muscular atrophy with respiratory distress type 1 (SMARD1).

SMARD1, also known as distal spinal muscular atrophy 1 (DSMA1) or distal hereditary motor neuropathies type 6 (dHMN6), is a rare autosomal recessive motor neuron disorder that affects infants and is characterized by diaphragmatic palsy, distal muscular weakness, and muscle atrophy. The disease is caused by mutations in the gene encoding immunoglobulin μ-binding protein 2 (*IGHMBP2*).

'Distal' SMA (DSMA1) is distinguished from 'proximal' autosomal recessive spinal muscular atrophy (SMA) by the primary muscles involved. Like the *SMN1* gene, which is mutated in SMA, *IGHMBP2* colocalises with the RNA-processing machinery in both the cytoplasm and the nucleus. *IGHMBP2* and *SMN1* share common functions important to motor neuron maintenance and integrity in mammals. *IGHMBP2* is the second gene found to be defective in SMA (Grohmann et al. 2001).

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 *GHMBP2* exon numbering used in this P058-A4 IGHMBP2 product description is the exon numbering from the RefSeq transcript NM_002180.2, which is identical to the LRG_250 sequence. The exon numbering and NM_ sequence used have been retrieved on 07/2020. 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 P058-A4 IGHMBP2 contains 25 MLPA probes with amplification products between 131 and 375 nucleotides (nt). This includes 16 probes for the *IGHMBP2* gene, one probe for each exon of the gene and two probes for exon 1. 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 spinal muscular atrophy with respiratory distress type 1. 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

Copy number status	Dosage quotient
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 *IGHMBP2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P058 IGHMBP2.
- 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.

***IGHMBP2* mutation database:** https://databases.lovd.nl/shared/genes/IGHMBP2. 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 *IGHMBP2* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P058-A4 IGHMBP2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	IGHMBP2
64-105	Control fragments – see table in probemix content section for more information		
131 *	Reference probe 00797-L25925	5q31	
136	**IGHMBP2 probe** 10471-L11024		**Exon 6**
142	**IGHMBP2 probe** 10490-L11043		**Exon 15**
148	Reference probe 00798-L00316	13q32	
154 *	Reference probe 08375-L08229	15q24	
160	**IGHMBP2 probe** 10482-L11035		**Exon 12**
166	**IGHMBP2 probe** 10469-L11022		**Exon 5**
172	**IGHMBP2 probe** 10475-L12572		**Exon 10**
178 *	Reference probe 08599-L28166	17p11	
184	**IGHMBP2 probe** 10473-L11026		**Exon 8**
190	**IGHMBP2 probe** 10484-L11037		**Exon 13**
196	**IGHMBP2 probe** 10466-L11019		**Exon 4**
208	Reference probe 10080-L10504	8q22	
214	**IGHMBP2 probe** 10455-L11008		**Exon 1**
229	Reference probe 10889-L11559	9p21	
250	**IGHMBP2 probe** 21086-L11027		**Exon 9**
256	**IGHMBP2 probe** 10479-L11032		**Exon 11**
274	**IGHMBP2 probe** 10458-L11011		**Exon 1**
284	**IGHMBP2 probe** 10472-L11025		**Exon 7**
297	Reference probe 05716-L05155	4p12	
310	**IGHMBP2 probe** 10463-L11016		**Exon 3**
319	**IGHMBP2 probe** 10488-L11041		**Exon 14**
338	Reference probe 09260-L11427	7q22	
359	**IGHMBP2 probe** 10460-L11013		**Exon 2**
375	Reference probe 16444-L18897	18q21	

* New in version A4.

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

Table 2. *IGHMBP2* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	IGHMBP2 exon[a]	Ligation site NM_002180.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*103-105 (Exon 1)*		
214	10455-L11008	Exon 1	45-46	GTCCGCTGTAAC-ACCGGCCCGGCG	0.1 kb
274	10458-L11011	Exon 1	128-129	TGTGGAGAGCTT-CGTGACCAAGCA	2.1 kb
359	10460-L11013	Exon 2	243-244	GGCGTGTGTTTG-CTGAAGCTGCAG	2.1 kb
310	10463-L11016	Exon 3	489-490	TTGAGCTTGGAC-CGAGAGAATTCC	0.3 kb
196	10466-L11019	Exon 4	596-597	CCCAGCCTCCTC-ACTCATAGAAGT	2.9 kb
166	10469-L11022	Exon 5	741-742	GCCATCATCCAT-GGACCTCCTGGC	3.3 kb
136	10471-L11024	Exon 6	854-855	CGTGGACAATCT-GGTGGAGCGCCT	3.0 kb
284	10472-L11025	Exon 7	1144-1145	CGGCAAACGTGG-TCCTTGCAACAA	11.4 kb
184	10473-L11026	Exon 8	1204-1205	TGCCCGAGAGCT-ACTTCGACGTGG	4.2 kb
250	21086-L11027	Exon 9	1444-1445	TGCACCAGGCTA-TCATGCGCTGGG	0.4 kb
172	10475-L12572	Exon 10	1551-1552	GCCACAGAAGAG-ACGGGTGTGCCC	0.7 kb
256	10479-L11032	Exon 11	1692-1693	GACGCTGGTGTT-CCAGCCCGTGAC	0.9 kb
160	10482-L11035	Exon 12	1813-1814	TCCAAGGCCGAG-AGAAGGAGGCCG	1.0 kb
190	10484-L11037	Exon 13	1970-1971	CCATGCATTTTT-GAAGACCCTGGT	1.9 kb
319	10488-L11041	Exon 14	2778-2779	GCCGCCGTTAAG-GCTGATAACACC	1.8 kb
142	10490-L11043	Exon 15	3418-3419	CAGGGCCTCAAA-CTTGAAGTCACT	
		stop codon	*3082-3084 (Exon 15)*		

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

P021 SMA — Spinal Muscular Atrophy (SMA), to determine *SMN1* and *SMN2* copy number changes (patients).

P060 SMA carrier — Spinal Muscular Atrophy (SMA) carrier, to determine *SMN1* and *SMN2* copy number changes of exon 7 and 8 only.

References

- Grohmann K et al. (2001). Mutations in the gene encoding immunoglobulin µ-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. *Nat Genet*, 29(1), 75-77.
- 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 P058 IGHMBP2

- Jędrzejowska M et al. (2014). Severe phenotypes of SMARD1 associated with novel mutations of the IGHMBP2 gene and nuclear degeneration of muscle and Schwann cells. *Eur J Paediatr Neurol*, 18(2), 183-192.
- Stalpers XL et al. (2013). Clinical and mutational characteristics of spinal muscular atrophy with respiratory distress type 1 in The Netherlands. *Neuromuscul Disord*, 23(6), 461-468.

P058 Product history	
Version	*Modification*
A4	Three reference probes have been replaced.
A3	One reference probe has been removed and one replaced. In addition one probe length has been adjusted.
A2	Two reference probes have been replaced and new control fragments (QDX2) have been included.
A1	First release.

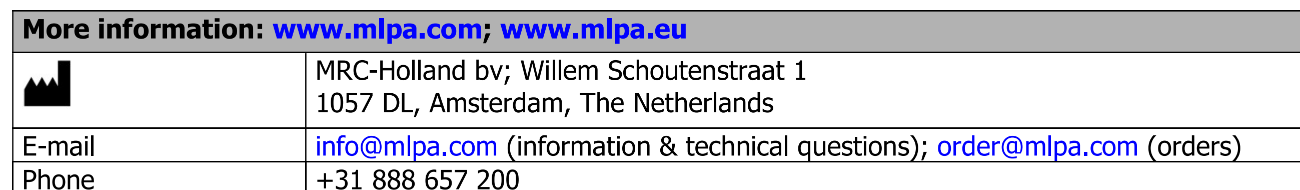
Implemented changes in the product description

Version A4-01 — 28 August 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).

Version 10 – 17 November 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, new picture included).
- Changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various textual changes throughout the document.

Version 09 – 12 August 2015 (54)
- Various minor textual changes.
- Figure(s) based on the use of old MLPA buffer (replaced in December 2012) removed.
- “Peak area” replaced with “peak height”.

Version 08 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.



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