Product Description SALSA® MLPA® Probemix P460-A1 SMA

To be used with the MLPA General Protocol.

Version A1. For complete product history see page 9.

Catalogue numbers:
- **P460-025R:** SALSA MLPA Probemix P460 SMA, 25 reactions.
- **P460-050R:** SALSA MLPA Probemix P460 SMA, 50 reactions.
- **P460-100R:** SALSA MLPA Probemix P460 SMA, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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.

This SALSA MLPA probemix is for basic research! Interpretation of results obtained with this product can be complicated.

Although the great majority of spinal muscular atrophy (SMA) carriers can be detected by copy number analysis of the *SMN1* exon 7 sequence, some carriers remain undetected. These include carriers with (1) a defective *SMN1* allele due to a point mutation in the *SMN1* gene or a copy number change of exons 1-6 or 8, and (2) individuals that have two *SMN1* copies on one allele and none on the other allele, the so-called "silent carriers". The P460 probemix may help to identify the latter group.

In most populations, approximately 6.3-15.5% of the individuals have two *SMN1* copies on a single chromosome 5 strand, of which 0.07-0.19% is a silent carrier. In the African-American population this percentage is even as high as 47.2%, of which 0.41% is a silent carrier (Hendrickson et al 2009).

Luo et al. (2014) reported that a specific *SMN1* haplotype block is present in a large percentage of Ashkenazi Jews who carry an *SMN1* duplication. This haplotype was also identified on *SMN1* duplication alleles in other ethnic groups, but in lower percentages.

In this P460 probemix, probes for the rare allele of two polymorphisms of this *SMN1* haplotype are included: g.27134T>G and g.27706-27707delAT. These probes do not give a signal on the great majority of DNA samples tested. When they do give a signal, it is usually on samples that contain three or four *SMN1* copies. In case these two probes give a signal on a sample that has only two copies of *SMN1*, there is an increased risk, depending on the ethnic background, that the person tested is a silent SMA carrier who carries two *SMN1* copies on one allele and none on the other.

As compared to the widely used MLPA probemix P060 SMA, probes for the rare allele of two *SMN1* polymorphisms are present in this P460 probemix. Furthermore, the length of the SMN1 exon 8 probe is different and the SMN2 exon 8 probe has been removed. In addition, three reference probes have been replaced and one reference probe was added.

Please be aware that presence of these polymorphisms may be indicative of a duplication (silent carrier) in the Ashkenazi Jew population, but the significance is less clear in other populations (see Luo et al., 2014).

General Information: The SALSA MLPA Probemix P460 SMA is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SMN1* and *SMN2* genes, which are associated with Spinal Muscular Atrophy (SMA). This Probemix can also be used to detect the presence of two polymorphisms, g.27134T>G and g.27706-27707delAT, for haplotype identification.

Spinal Muscular Atrophy (SMA) is a neuromuscular disorder characterised by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. SMA is the second most common lethal autosomal recessive disorder in Caucasians, after cystic fibrosis. SMA is usually divided into three clinical groups. Patients with type I SMA (MIM# 253300) show onset at birth or before six months, and usually die of respiratory insufficiency within two years. Type I SMA patients are never able to sit or walk. Patients with type II SMA (MIM# 253550) show onset after 6 months. They can sit but are never able to walk unaided, and their life expectancy is significantly reduced. Type III SMA (MIM# 253400) patients show the first symptoms after 18 months and are able to stand and walk, but often become wheelchair-bound during youth or adulthood.

There are two (highly-similar) genes playing a pivotal role in SMA: *SMN1* and *SMN2*. The telomeric *SMN1* and the centromeric *SMN2* genes are located in a complicated inverted repeat area spanning ~500 kb on chromosome 5q13.2. This area displays high instability, leading to frequent deletions and gene conversions. Most individuals have two copies each of *SMN1* and *SMN2*. The *SMN1* and *SMN2* genes, each having nine exons (exons 1, 2a, 2b, and 3-8), can only be distinguished by two single nucleotide differences: one in exon 7 and one in exon 8. The exon 8 difference has no effect on the transcript, however, the exon 7 difference disrupts a putative exonic splicing enhancer in *SMN2*. Only 10-15% of the *SMN2* transcripts are functional. This SALSA MLPA probemix P460 SMA detects the copy number of exon 7 and exon 8 of the *SMN1* gene and exon 7 of the *SMN2* gene.

Absence of any functional *SMN1* copy results in insufficient amounts of full length transcripts. More than 95% of SMA patients show homozygous deletion of at least exon 7 of the telomeric *SMN1* gene. The great majority of SMA carriers can be identified by the presence of only a single *SMN1* exon 7 copy. The one copy frequency in the US is estimated to be 1:37 for Caucasians, 1:46 for Ashkenazi Jews, 1:56 for Asians, 1:91 for African-Americans and 1:125 for Hispanics (Hendrickson et al. 2009). The SMN2 copy number is very variable with only 60-70% of individuals having two copies.

The presence of *SMN2* results in a small amount of full length transcripts. Establishing the number of the *SMN2* copy number is important for SMA patients: the more *SMN2* copies a patient has, the more functional SMN protein is produced and the milder the SMA phenotype is. The majority of type I SMA patients carry a homozygous deletion of *SMN1* and a normal or reduced number of *SMN2* copies. The majority of the type II and III SMA patients show homozygous absence of *SMN1* and an increased number of *SMN2* copies (3–4 copies). A homozygous deletion of *SMN2* is found in about 5% of healthy individuals. This has no clinical phenotype when at least one functional *SMN1* copy is present. Healthy individuals lacking *SMN1* but having five or more *SMN2* copies have been described.

More information on Spinal Muscular Atrophy is available at http://www.ncbi.nlm.nih.gov/books/NBK1352/.

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited MLPA 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/

Probemix content: The SALSA MLPA Probemix P460-A1 SMA contains 23 MLPA probes with amplification products between 131 and 331 nt. This includes 3 probes for the *SMN1* and *SMN2* genes. Furthermore, it also contains 2 probe(s) specific for the polymorphisms g.27134T>G and g.27706-27707delAT, which will only generate a signal when a polymorphism is present. In addition, 18 reference probes are included and detect 18 different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.com).

This Probemix contains nine quality control fragments generating amplification products between 64 and 121 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 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 SMA. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

The choice of reference samples is important for the correct determination of the *SMN1* and *SMN2* copy numbers. MRC-Holland is not able to provide reference DNA samples. One reason is that MLPA reactions on all samples, including reference samples, should be done on DNA extracted by the same method, as stated above. It is strongly advised to first make a selection of suitable reference samples with known copy numbers before SMA testing is started. One method of doing this is to test a number (e.g. 16) of healthy individuals. Identification of samples having two copies of both *SMN1* and *SMN2* should usually be simple as in most populations these will constitute the great majority of the samples. The SD042 sample DNA can help in identifying suitable reference DNA samples. Please note that in some populations, such as African-Americans, the number of individuals with a total of three *SMN1* copies may be almost identical to those with two copies (Hendrickson et al. 2009).

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and 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. Sample ID numbers NA00232, NA03815, HG01773, NA19984, NA20294, HG02882 and GM19235 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample(s) to detect specific copy number changes in *SMN1* and *SMN2* (see table below). The quality of cell lines can change, therefore samples should be validated before use.

SMN1 and _SMN2_ copy number of selected Coriell cell lines and SD042 sample DNA.

Coriell #	_SMN1_ exon 7 copy number	_SMN1_ exon 8 copy number	_SMN2_ exon 7 copy number	g.27134T>G copy number	g.27706-27707delAT copy number
NA00232	0	0	2	0	0
NA03815	1	1	1	0	0
HG01773	1	1	4	0	0
NA19984	2	2	1	1	1
NA20294	3	3	1	1	1
HG02882	3	3	1	2	2
GM19235	4	4	0	3	3
SD042	2	2	2	1	1

As an alternative, the SD042 sample DNA is provided with this probemix. This sample DNA can be used in data binning in the fragment analysis, as a positive control for the polymorphism-specific probes and for selection of suitable reference samples that have two copies each of *SMN1* and *SMN2*.

SALSA Binning DNA SD042: The SD042 Binning DNA provided with this Probemix can be used as Binning DNA sample for binning of the g.27134T>G and g.27706-27707delAT polymorphism-specific probes (143 nt SMN1 probe S0938-L26163 g.27134T>G and 148 nt SMN1 probe S0961-L25586 g.27706-27707delAT). SD042 Binning DNA is a mixture of female genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD042 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s), as for this purpose true polymorphism positive patient samples or cell lines should be used. It is strongly advised to use DNA sample and reference DNA samples extracted with the same method and derived from the same source of tissue. For further details, please consult the SD042 Binning DNA product description provided. **This product is for research use only (RUO).**

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 all reference probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the test 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 or pseudo-autosomal chromosomes:

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

- False positive results: Please note that detected abnormalities 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. 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.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Description of the SMN probes in P460-A1 SMA

- The **SMN1 Exon 7 probe 14919-L17081** (183 nt) is the most important probe in this probemix. This probe is specific for *SMN1* and gives no significant signal on *SMN2*. The probe has its ligation site at the C-to-T transition in exon 7, which is a site that affects RNA splicing. The presence of *SMN1* sequences (cytosine at the ligation site) in the sample results in a peak at 183 nt. This probe can be used to determine the *SMN1* copy number, which is important for the determination of a SMA carrier status. Of the 5q13-linked SMA patients, 96.4% show a homozygous deletion of *SMN1* exon 7 and 8, or exon 7 only. The remaining 3.6% present a compound heterozygosity with a subtle mutation on one chromosome and a deletion/gene conversion on the other. Such a subtle mutation is likely not to be detected by this P460 SMA MLPA assay and should be identified by sequencing. In a very small number of patients, the *SMN1* defect is a copy number change of *SMN1* exons 1-6 which may be detectable with the P021 SMA MLPA probemix (Arkblad et al. 2006).

- The **SMN1 Exon 8 probe S0960-L25957** (154 nt) is able to distinguish *SMN1* from *SMN2* at exon 8 (G-to-A transition). The signal of this probe indicates the copy number of *SMN1* exon 8. In approximately 95% of the samples, the copy number detected by the *SMN1* exon 7 probe and the *SMN1* exon 8 probe is identical. This *SMN1* exon 8 probe cannot be used to quantify *SMN1* copies, as an exon 8 mutation will still result in a functional protein. Only the SMN1 exon 7 probe should be used to determine the *SMN1* copy number! In the majority of the remaining 5% of the samples, gene conversion between *SMN1* and *SMN2* has resulted in a chimeric gene containing the *SMN1* exon 7 sequence and the *SMN2* exon 8 sequence. Such a hybrid gene results in a functionally identical protein as the SMN1 protein.

- The **SMN2 Exon 7 probe 14921-L17083** (282 nt) shows the *SMN2* copy number which is important for SMA patients, but which has no influence on SMA carrier status. The *SMN2* copy number is much more variable than the *SMN1* copy number. Approximately 5% of individuals lack the *SMN2* gene.

- The **SMN1 Intron 7 S0938-L26163 and Exon 8 S0961-L25586 probes** (143 and 148 nt) detect the rare allele of the g.27134T>G and g.27706-27707delAT polymorphisms, respectively. The probes have been included to increase the detection rate of so-called "silent carriers": an individual with two *SMN1* copies on one chromosome and none on the other. These probes do not generate a signal in the great majority of the samples. Most samples in which at least one of these probes *does* give a signal have three or more *SMN1* copies, as determined by the *SMN1* exon 7 probe. These individuals are unlikely to be a SMA carrier as they usually have at least one functional *SMN1* copy on each chromosome. However, individuals that are (1) positive for at least one of these two polymorphism specific probes **AND** (2) have only two *SMN1* copies (the latter determined by the 183 nt SMN1 exon 7 probe) have an increased chance, depending on their ethnicity, of being a "silent carrier" as they may have both copies on the same allele. See Luo et al. (2014).

The summary of these findings and what they may mean for carrier/patient status can be found in the Table on the next page.

Overview of expected results and the corresponding most likely conclusion

Finding	Most likely conclusion	Explanation
SMA symptoms - 183 nt *SMN1* exon 7: 0 copies. - 154 nt *SMN1* exon 8: 0 copies.	SMA patient	*SMN1* is absent, as no copies of the distinct *SMN1* exon 7 are present. The absence of both *SMN1* exon 8 copies can be seen as an extra confirmation.
SMA symptoms - 183 nt *SMN1* exon 7: 0 copies. - 154 nt *SMN1* exon 8: 1 or more copies.	SMA patient	*SMN1* is absent, as no copies of the determining *SMN1* exon 7 sequence are found. Due to gene conversion, one or more copies of the characteristic *SMN1* exon 8 sequence appear to have become incorporated in the *SMN2* gene.
SMA symptoms - 183 nt *SMN1* exon 7: 1 copy.	SMA patient	If the patient has SMA symptoms, but one copy of *SMN1* exon 7 is present, the patient may belong to the 3.6% group presenting compound heterozygosity. Sequencing might reveal a defect in the remaining *SMN1* copy.
No SMA symptoms - 183 nt *SMN1* exon 7: 1 copy. - 154 nt *SMN1* exon 8: 1 copy.	SMA carrier	One copy of *SMN1* is absent, making the person a carrier. The absence of one copy of the *SMN1* exon 8 sequence can be seen as an extra confirmation.
No SMA symptoms - 183 nt *SMN1* exon 7: 1 copy. - A: 154 nt *SMN1* exon 8: copies > 1. - B: 154 nt *SMN1* exon 8: copies = 0.	SMA carrier	One copy of *SMN1* is absent, making the person a carrier. A: due to gene conversion, one (or more) copies of the characteristic *SMN1* exon 8 have become incorporated in the *SMN2* gene. B: an *SMN2* exon 8 copy has replaced the characteristic *SMN1* exon 8 copy.
No SMA symptoms - 183 nt *SMN1* exon 7: 2 copies. - 143 and/or 148 nt polymorphism probes: no signals	Most likely not a SMA carrier	Most likely this person is not a carrier. However, there is a possibility that both *SMN1* copies lie on one chromosome. If there is reason to believe that the person is a carrier (i.e. child is SMA-patient), he/she may belong to the 4% of carriers where this is indeed the case.
No SMA symptoms - 183 nt *SMN1* exon 7: 2 copies. - 143 nt and/or 148 nt polymorphism probes: signals present	Increased chance on being SMA carrier	There is a higher chance that both *SMN1* copies lie on one chromosome (likelihood will vary per population). If this is indeed the case, there is a 50% chance that not a single *SMN1* copy is passed on by this parent to the offspring. In this case, it is recommended to test the partner in order to determine the risk of conceiving an affected child.

False negative results SMA carrier screening

Be aware that for carrier screening, false negative results can be obtained. This MLPA test does not detect deleterious point mutations in the *SMN1* gene, does not detect all rearrangements of the *SMN1* gene that can inactivate the gene copy, and does not identify all cases in which 2 *SMN1* copies are located on the same chromosome and none on the other.

False positive results SMA carrier screening

Please note that individual MLPA probes can be affected differently by changes in experimental procedures or impurities in samples. Highly unlikely results such as an unusual high frequency of *SMN1* exon 7 loss (carrier) or *SMN1* exon 7 gain, without loss or gain of the exon 8 probe in most of these samples, should be treated with caution.

Limitations of the procedure:

- 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: Detected copy number changes 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.

SMA mutation database: http://grenada.lumc.nl/LSDB_list/lsdbs/SMN1. We strongly encourage users to deposit positive results in the LOVD SMN1 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 SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P460-A1 SMA

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)		
		Reference	*SMN1*	*SMN2*
64-105	Control fragments – see table in probemix content section for more information			
131	Reference probe 00797-L25925	5q31		
136	Reference probe 18457-L23634	6q24		
143 §	**SMN1 probe** S0938-L26163		g.27134T>G	
148 §	**SMN1 probe** S0961-L25586		g.27706-27707delAT	
154	**SMN1 probe** S0960-L25957		**Exon 8**	
163	Reference probe 02291-L17086	3p14		
172	Reference probe 02978-L17087	4q25		
183	**SMN1 probe** 14919-L17081		**Exon 7**	
191	Reference probe 00559-L17088	11q22		
200	Reference probe 00976-L17298	11p13		
208	Reference probe 12490-L17096	1q32		
228	Reference probe 14498-L17101	20p12		
237	Reference probe 02334-L17301	12q23		
245	Reference probe 14293-L17100	15q13		
255	Reference probe 13128-L17099	9q34		
264	Reference probe 07630-L17091	10q26		
272	Reference probe 14361-L17098	4q35		
282	**SMN2 probe** 14921-L17083			**Exon 7**
292	Reference probe 18491-L23716	3q12		
301	Reference probe 12783-L13918	2q12		
311	Reference probe 06425-L17092	6p22		
321	Reference probe 01042-L17093	8q24		
331	Reference probe 01043-L17094	8q13		

§ Polymorphism-specific probe. This probe will not generate a signal in the majority of samples. The 143 nt and 148 nt probes will only generate a signal when the g.27134T>G and g.27706-27707delAT polymorphisms are present, respectively.

Note: The exon numbering used in this P460-A1 SMA product description and in the P460-A1 SMA lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1, 2a, 2b, and 3-8). This exon numbering is different from the NCBI reference sequences for *SMN1* and *SMN2*. Please notify us of any mistakes: info@mlpa.com.

Table 2. SMN1 and SMN2 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene Exon	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
183 ∧	14919-L17081	*SMN1* exon 7	TTACAGGGTTTC-AGACAAAATCAA	0.1 kb
143 #	S0938-L26163	g.27134T>G	GAACTTTTCAAC-TGTTCAAAAACA	0.5 kb
154 +	S0960-L25957	*SMN1* exon 8	CCACCCCCACCC-CAGTCTTTTACA	0.1 kb
148 #	S0961-L25586	g.27706-27707delAT	TTACTGGACTCT-TTTGAAAAACCA	> 100 kb
282	14921-L17083	*SMN2* exon 7	TTACAGGGTTTT-AGACAAAATCAA	

∧ One copy in most carriers; none in most patients.
+ Confirms *SMN1* exon 7 results in 95% of samples.
# These probes do not generate a signal in the majority of samples. Please be aware that presence of these polymorphisms may be indicative of a duplication (silent carrier) in particular in the Ashkenazi Jew population. Screening of family members may be helpful in detecting silent carriers.

Note: The exon numbering used in this P460-A1 SMA product description and in the P460-A1 SMA lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1, 2a, 2b, and 3-8). This exon numbering is different from the NCBI reference sequences for *SMN1* and *SMN2*. 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 (patients).
- P060 SMA: Spinal Muscular Atrophy (SMA), to determine *SMN1* and *SMN2* copy number (carriers).
- P058 IGHMBP2: Autosomal recessive distal spinal muscular atrophy 1 (DSMA1), gene included *IGHMBP2*.

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Selected publications using SALSA MLPA in SMA research

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P460 Product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description

Version A1-01 – 16 May 2018 (01P)
- Product description restructured and adapted to a new template.

Version 08 (55) – 02 May 2018
- Minor textual change (percentages of silent carriers adjusted).

Version 07 (55) – 14 March 2018
- HG02051 removed from Table 1 as the exact SMN1 exon 8 and SMN2 exon 7 copy number is not clear.
- SMN2 exon 7 copy number of NA03815 corrected in Table 1.
- Header corrected in the third column of Table 1.
- Reference details updated.
- Various minor textual and layout changes.

Version 06 (55) – 17 July 2017
- Various minor textual changes.
- Table 1 with positive samples included.

Version 05 (55) – 14 February 2016
- Product description adapted to a new lot (lot number added, new picture included).
- Adjustments on confirmation of results on page 2.

Version 04 (55) – 23 October 2015
- Various minor textual changes.
- Warning added for probes detecting SNP g.27134T>G and SNP g.27706-27707delAT, and the significance in other than the Ashkenazi Jewish population.

Version 03 (54) – 16 July 2015
- Not applicable, new product.

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