Product Description

SALSA® MLPA® Probemix P460-A1 SMA (Silent) Carrier

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 (Silent) Carrier, 25 reactions
- **P460-050R:** SALSA® MLPA® Probemix P460 SMA (Silent) Carrier, 50 reactions
- **P460-100R:** SALSA® MLPA® Probemix P460 SMA (Silent) Carrier, 100 reactions

SALSA® MLPA® Probemix P460 SMA (Silent) Carrier (hereafter: P460 SMA (Silent) Carrier) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

P460 SMA (Silent) Carrier can be used in combination with:

- SALSA® Reference Selection & Binning DNA SD084 (Cat. No: SD084)

Volumes and ingredients

Volumes			Ingredients
P460-025R	**P460-050R**	**P460-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. A Safety Data Sheet (SDS) is not required for this product: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Certificate of Analysis
Information regarding 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.

Please note: This product should not be used to determine *SMN2* copy numbers for the purpose of determining disease prognosis or eligibility for SMA therapeutics. For the most reliable *SMN2* copy number detection, use SALSA MLPA Probemix P021 SMA (from version B1 onwards).

General information

SALSA® MLPA® Probemix P460 SMA (Silent) Carrier is a **research use only (RUO)** assay for the detection of 1. copy number changes of exons 7 and 8 of the *SMN1* gene for carrier testing and 2. the presence of the g.27134T>G (rs143838139; NM_000344.4:c.*3+80T>G (MANE Select)) and g.27706-27707delAT (rs200800214; NM_000344.4:c.*211_*212del (MANE Select)) polymorphisms in *SMN1* that are a risk factor for silent carriership of SMA in genomic DNA isolated from human peripheral whole blood specimens. Note that this probemix cannot determine the zygosity of the mentioned polymorphisms.

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 (OMIM# 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 (OMIM# 253550) show onset after six months. They can sit but are never able to walk unaided, and their life expectancy is significantly reduced. Type III SMA (OMIM# 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*, both consisting of nine exons (exons 1, 2a, 2b, and 3-8). The *SMN1* and *SMN2* genes 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 splicing in *SMN2* most of the time leading to loss of functionality. Only 10-15% of the *SMN2* transcripts are functional. P460 SMA (Silent) Carrier detects the copy number of exons 7 and 8 of the *SMN1* 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. Individuals with only one functional *SMN1* copy are carriers of the disease. 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).

Although the great majority of 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” (2+0 genotype). The P460 probemix increases the detection rate of 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% are 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) has 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. Detection of the SMN1 polymorphisms g.27134T>G and g.27706-27707delAT can aid in identifying this haplotype and thereby silent carriers.

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

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE

Exon numbering
The *SMN1* and *SMN2* exon numbering used in this P460 SMA (Silent) carrier product description and lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1, 2a, 2b, and 3-8), as mentioned in OMIM: *SMN1* (#600354) and *SMN2* (#601627). This exon numbering is different from the MANE select transcripts: NM_000344.4 (*SMN1)* and NM_017411.4 (*SMN2*). As changes to the databases can occur after release of this product description, the NM_ sequences may not be up-to-date.

Probemix content
P460-A1 SMA (Silent) Carrier contains 23 MLPA probes with amplification products between 131 and 331 nucleotides (nt). This includes three probe(s) for the for the *SMN1* and *SMN2* genes. Furthermore, this probemix also contains two probe(s) specific for the g.27134T>G (rs143838139; NM_000344.4:c.*3+80T>G (MANE Select)) and g.27706-27707delAT (rs200800214; NM_000344.4:c.*211_*212del (MANE Select)) mutations which will only generate a signal when the mutation is present. In addition, 18 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).

- The **SMN1 Exon 7 probe 14919-L17081** (183 nt) is the most important probe as it can be used to determine *SMN1* copy number. This probe is specific for *SMN1* and will give no significant signal on *SMN2*. The probe has its ligation site at the C-to-T transition in exon 7, which is the site that affects RNA splicing in *SMN2*.

- The **SMN1 Exon 8 probe S0960-L25957** (154 nt) is able to distinguish between *SMN1* and *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 and exon 8 probes is identical. This *SMN1* exon 8 probe cannot be used to quantify the number of *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 samples, gene conversion between *SMN1* and *SMN2* has resulted in a hybrid gene containing the *SMN1* exon 7 sequence and the *SMN2* exon 8 sequence. Such a hybrid gene results in a functionally identical protein to the SMN1 protein.

- The **SMN2 Exon 7 probe 14921-L17083** (282 nt) identifies the *SMN2* exon 7 copy number. (Determining *SMN2* copy number for the purpose of prognosis, using SALSA MLPA Probemix P021 SMA is recommended.)

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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all reference probes over the experiment. Note that the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.

Required specimens
Extracted DNA from human peripheral whole blood specimens, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from 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 (www.mrcholland.com).

Correct determination of the *SMN1* copy numbers is completely dependent on the reference sample selection. 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. Selection of suitable reference samples with known copy numbers should always be performed 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* genes and no copies of the *SMN1* polymorphisms g.27134T>G and g.27706-27707delAT should usually be simple as in most populations these will constitute the great majority of the samples. The SD084 Reference Selection & Binning 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).

SALSA® Reference Selection & Binning DNA SD084
The SALSA® Reference Selection & Binning DNA SD084 provided with this probemix can be used for two distinct functions. First, it can be used for binning of the two polymorphism-specific probes (SMN1 probe S0938-L26163 g.27134T>G polymorphism and SMN1 probe S0961-L25586 g.27706-27707delAT polymorphism) during data analysis, and second for the selection of suitable reference samples before an experiment is started. Suitable reference samples contain two copies each of *SMN1* and *SMN2*, but do not contain the two polymorphisms. SD084 is a mixture of human female genomic DNA from healthy individuals and a titrated amount of plasmid DNA that contains the target sequence detected by the above mentioned probes.

For the purpose of binning, inclusion of one reaction with 5 µl SD084 Reference Selection & 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, SD084 Reference Selection & 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). SD084 Reference Selection & Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of polymorphism signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue.

The selection of suitable reference DNA samples that can be used with P460 SMA (Silent) Carrier is complicated, due to the high frequency of gene conversions and CNVs in the healthy population. For the purpose of facilitating the selection of suitable reference DNA samples (containing two copies each of *SMN1* and *SMN2* and not containing the two polymorphisms) from your own sample collection, the SD084 Reference Selection & Binning DNA sample can also be used. SD084 Reference Selection & Binning DNA should only be used for initial experiments on DNA samples from healthy individuals with the intention to identify suitable reference samples. SD084 should not be used as a reference sample in subsequent experiments. For further details, consult the SD084 Reference Selection & Binning DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Positive control DNA samples
See the section "Positive samples" on the P460 product page on our website.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net 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 reference 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/gain	1.30 < FR < 1.65
Copy numbers ≥4	FR > 1.75#
Ambiguous copy number	All other values

*There is a high sequence similarity between *SMN1* and *SMN2* exon 7 and 8. In case of a homozygous deletion of one of the probe targets, a small background signal can still be visible. This is caused by nonspecific binding of these probes to the corresponding exon of the homologous gene. This background signal might be displayed as an intra ratio percentage (orange box in the ratio chart) instead of a Final Ratio (see our support portal for more details).

#SMN2 probes in this probemix cannot reliably distinguish between 4 or more copies. A final ratio of > 1.75 for SMN2 probes should be interpreted as 4 or more copies. SALSA MLPA Probemix P021 SMA can be used for a more accurate *SMN2* copy number determination.

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged). Please note that Coffalyser.Net 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.

The above mentioned FR values do not apply to the polymorphism-specific probes. The peaks of the polymorphism-specific probes are expected to be absent in the majority of samples tested and therefore their standard deviation cannot be determined. Clear signal (at least 10% of the median peak height of all reference probes in that sample) for one of these probes indicates that the mutation is present.

- 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 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

- 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.
- Be aware that for carrier screening, false negative results can be obtained as not all silent carriers can be detected using the P460 probemix. MLPA is not able to determine whether the two *SMN1* copies are on the same or on different chromosomes but can, depending on ethnicity, increase the detection rate of silent carriers. As mentioned above, the carrier screening in certain populations such as African-Americans and possibly other individuals of African descent may be compromised by a higher frequency of individuals with two or more *SMN1* copies on one chromosome.
- SMN2 probes in this probemix cannot reliably distinguish between 4 or more copies.

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 in sequence data 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. SALSA MLPA probemixes P021 SMA and P060 SMA Carrier cannot be used for confirmation of results.

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 https://varnomen.hgvs.org.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 1. P460-A1 SMA (Silent) Carrier

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*SMN1*	*SMN2*
64-105	Control fragments – see table in probemix content section for more information			
131	Reference probe 00797-L25925	5q		
136	Reference probe 18457-L23634	6q		
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	3p		
172	Reference probe 02978-L17087	4q		
183 ∫	**SMN1 probe** 14919-L17081		**Exon 7**	
191	Reference probe 00559-L17088	11q		
200	Reference probe 00976-L17298	11p		
208	Reference probe 12490-L17096	1q		
228	Reference probe 14498-L17101	20p		
237	Reference probe 02334-L17301	12q		
245	Reference probe 14293-L17100	15q		
255	Reference probe 13128-L17099	9q		
264	Reference probe 07630-L17091	10q		
272	Reference probe 14361-L17098	4q		
282 « ∫	**SMN2 probe** 14921-L17083			**Exon 7**
292	Reference probe 18491-L23716	3q		
301	Reference probe 12783-L13918	2q		
311	Reference probe 06425-L17092	6p		
321	Reference probe 01042-L17093	8q		
331	Reference probe 01043-L17094	8q		

[a] See section Exon numbering on page 3 for more information.

Note: The exon numbering used in this P460-A1 SMA (Silent) Carrier product description and in the P460-A1 SMA (Silent) Carrier 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@mrcholland.com.

§ 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.

≈ Incomplete ligase inactivation can lead to a peak at ~148 nt even when the polymorphism is not present. Results obtained should be treated with caution when a signal for this probe is observed for all samples in an experiment.

« A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

∫ Due to a high sequence similarity between *SMN1* and *SMN2* exon 7 and 8, a small background signal can be visible in homozygous deletion samples (see section Interpretation of Results for more information).

Table 2. SMN probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b] NM_017411.4 (*SMN2*), NM_000344.4 (*SMN1*)	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
282 ∫	14921-L17083	*SMN2* exon 7	857-858	TTACAGGGTTTT-AGACAAAATCAA	> 100 kb
183 Λ ∫	14919-L17081	*SMN1* exon 7	857-858	TTACAGGGTTTC-AGACAAAATCAA	0.1 kb
143 #	S0938-L26163	g.27134T>G	79 nt after exon 7 reverse	GAACTTTTCAAC-TGTTCAAAAACA	0.5 kb
148 #	S0961-L25586	g.27706-27707delAT	1112 . . 1115	TTACTGGACTCT-TTTGAAAAACCA	< 0.1 kb
154 + ∫	S0960-L25957	*SMN1* exon 8	1141-1140 reverse	CCACCCCCACCC-CAGTCTTTTACA	

[a] See section Exon numbering on page 3 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

Λ One copy in most carriers; none in most patients.

+ Confirms *SMN1* exon 7 results in in up to 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.
∫ Due to a high sequence similarity between *SMN1* and *SMN2* exon 7 and 8, a small background signal can be visible in homozygous deletion samples (see section Interpretation of Results for more information).

Related products

For related products, see the product page on our website.

References

- Alias L et al. (2018). Utility of two SMN1 variants to improve spinal muscular atrophy carrier diagnosis and genetic counselling. *Eur J Hum Genet.* 26:1554-1557.
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- Luo M. et al. (2014) An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy.*Genet Med*. 16:149-156.

- Prior TW et al. (2020). Characterization of Reference Materials for Spinal Muscular Atrophy Genetic Testing: A Genetic Testing Reference Materials Coordination Program Collaborative Project. *J Mol Diagn*. 23:103-110.
- 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 P460 SMA (Silent) Carrier

- Vitello GA et al. (2020). Possible implication of undescribed SMN1-SMN2 genotype in chronic EMG-pattern of SMA with transitory acute denervation. *J Musculoskelet Neuronal Interact.* 20:610-613.
- Zhang J et al. (2020). Carrier screening and prenatal diagnosis for Spinal Muscular Atrophy in 13 069 Chinese pregnant women. *J Mol Diagn.* 22:817-822.

P460 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-10 – 15 June 2026 (05P) - Product description updated as P460 is for research use only from lot 0326 onwards. Version A1-09 – 04 August 2025 (05P) - Adapted to new template. - Comparison of MRC Holland SMA products table updated: (i) P060 is no longer a secondary application for neonatal patient screening, (ii) primary and secondary test changed to primary and secondary application, (iii) symbol 'not possible to detect' removed and (iv) legend 'suitable to detect' removed to align with the table under Related products in the product page. - Explanation of background signal corrected in footnote under table in Interpretation of Results section. - Warnings for background signal of probes 14921-L17083, 14919-L17081 and S0960-L25957 added. - Explanation of polymorphism-specific probe signal added to Interpretation of Results section. - Minor textual changes. Version A1-08 – 01 April 2025 (04P) - Comparison of MRC Holland SMA products table updated: 'patient detection confirmation' row removed, added footnote that P060 cannot be used to determine *SMN2* copy numbers for diagnostic purposes, added description of silent carriers, minor textual changes. Version A1-07 – 08 November 2024 (04P) - Reference to SALSA Reference Selection & Binning DNA SD084 removed from the intended purpose footnote. - In section Positive control DNA samples replaced 'GM19235' with 'NA19235'. - In section SALSA Reference Selection & Binning DNA SD084 replaced 'synthetic DNA' with 'plasmid DNA' and added "This product is for research use only (RUO)". Version A1-06 – 05 June 2024 (04P) - Added that the *SMN2* probes in this probemix cannot distinguish between 4 and >4 copies of *SMN2* throughout this Product Description. Updated table in the Interpretation of results section to reflect this. - Minor textual changes. Version A1-05 – 22 November 2023 (04P) - Clarification on the use of other MRC Holland SMA products added to section "Confirmation of results". - Reference to Coriell sample adjusted in Positive control DNA samples section.

- Recommendation for parental evaluation added to section “Interpretation of results”.

Version A1-04 – 11 July 2022 (04P)
- Table on Final ratios adjusted.
- Information about background signal in Interpretation of results section adjusted.

Version A1-03 – 7 June 2021 (04P)
- Probemix name changed from P460 SMA to P460 SMA (Silent) Carrier.

Version A1-02 - 23 February 2021 (04P)
- Product description rewritten and adapted to new template.
- P460-A1 is now CE -marked.
- Intended purpose adapted to new template.
- Three additional references added.
- New NM_ sequence for SMN1 and SMN2 implemented.
- Warning concerning incomplete ligase heat inactivation added to Table 1.
- Added a comparison table between MRC Holland SMA products.
- SD084 has replaced SD042. This is reflected in this product description.
- Warning added to Table 1 on the salt sensitivity of the *SMN2* Exon 7 probe.

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IVD	EUROPE* CE (until lot 0922)
RUO	EUROPE* (as of lot 0326) ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.