# Product Description SALSA® MLPA® Probemix P295-B3 SPRED1

To be used with the MLPA General Protocol.

**Version B3.** As compared to version B2, two reference probes have been replaced. For complete product history see page 6.

**Catalogue numbers:**
- **P295-025R:** SALSA MLPA Probemix P295 SPRED1, 25 reactions.
- **P295-050R:** SALSA MLPA Probemix P295 SPRED1, 50 reactions.
- **P295-100R:** SALSA MLPA Probemix P295 SPRED1, 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.

**General Information:** The SALSA MLPA Probemix P295 SPRED1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SPRED1* gene, which is associated with Legius syndrome.

Legius syndrome (also known as Neurofibromatosis Type 1-like syndrome; NFLS) is characterised by multiple café-au-lait spots. Other features may include an abnormally large head (macrocephaly) and unusual facial characteristics. The syndrome has many symptomatic similarities with Neurofibromatosis Type I (NF1). *SPRED1* (Sprouty-related EVH1 domain containing 1) is a member of the Sprouty family of proteins that regulate growth factor-induced activation of the Ras/MAP kinase cascade. Mutations in the *SPRED1* gene cause Legius syndrome.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK47312/.

**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/

**Probemix content:** The SALSA MLPA Probemix P295-B3 SPRED1 contains 38 MLPA probes with amplification products between 149 and 454 nt. This includes 23 probes for the *SPRED1* gene. Multiple probes for each of the seven exons (or probes in close proximity of the exon) of the *SPRED1* gene are included. Probes in the upstream region and several probes in intronic regions of the gene have also been included in order to facilitate the detection of breakpoints of intragenic rearrangements. In addition, 15 reference probes are included and detect 15 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, 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 Legius syndrome. 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 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. 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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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 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 |

- 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 (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.

**Limitations of the procedure:**

- In most populations, the major cause of genetic defects in the *SPRED1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P295 SPRED1.
- 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.

***SPRED1* mutation database:** https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db=SPRED1. 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 *SPRED1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

## Table 1. SALSA MLPA Probemix P295-B3 SPRED1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18) Reference | *SPRED1* |
|---|---|---|---|
| 64-105 | Control fragments – see table in probemix content section for more information | | |
| 149 | Reference probe 10129-L11734 | 18q11 | |
| 154 | **SPRED1 probe** 13044-L14227 | | **Exon 3** |
| 160 Ø | **SPRED1 probe** 13045-L14228 | | **Intron 4** |
| 166 | Reference probe 08421-L08304 | 11q22 | |
| 172 | **SPRED1 probe** 13046-L14229 | | **Exon 5** |
| 178 Ø | **SPRED1 probe** 13047-L14230 | | **Intron 2** |
| 185 Ø | **SPRED1 probe** 13048-L14231 | | **Intron 1** |
| 196 | **SPRED1 probe** 11017-L11686 | | **Exon 4** |
| 202 * | Reference probe 03709-L25988 | 9q22 | |
| 208 | **SPRED1 probe** 13049-L28437 | | **Exon 4** |
| 214 | **SPRED1 probe** 11015-L28438 | | **Exon 2** |
| 220 | Reference probe 12427-L13428 | 22q12 | |
| 230 | **SPRED1 probe** 13050-L14233 | | **Exon 1** |
| 238 | **SPRED1 probe** 11019-L11688 | | **Exon 6** |
| 247 | **SPRED1 probe** 11016-L11685 | | **Exon 3** |
| 257 | Reference probe 17408-L21394 | 3p21 | |
| 265 | **SPRED1 probe** 11013-L13179 | | **Upstream** |
| 274 Ø | **SPRED1 probe** 13051-L14234 | | **Intron 1** |
| 283 | Reference probe 08680-L08692 | 13q32 | |
| 292 | **SPRED1 probe** 13052-L14235 | | **Exon 6** |
| 301 | **SPRED1 probe** 11020-L11689 | | **Exon 7** |
| 310 * | Reference probe 12442-L13443 | 14q24 | |
| 319 | Reference probe 08048-L07829 | 5p15 | |
| 328 | **SPRED1 probe** 13053-L14236 | | **Exon 1** |
| 337 | Reference probe 09937-L12248 | 8q13 | |
| 346 | **SPRED1 probe** 11018-L11687 | | **Exon 5** |
| 355 | **SPRED1 probe** 13054-L14237 | | **Exon 2** |
| 364 | Reference probe 12989-L14146 | 19q13 | |
| 373 | Reference probe 10718-L11300 | 6p12 | |
| 382 | **SPRED1 probe** 13055-L14238 | | **Exon 7** |
| 391 | Reference probe 14043-L15641 | 7q36 | |
| 402 | **SPRED1 probe** 11021-L11690 | | **Exon 7** |
| 409 | **SPRED1 probe** 13056-L14239 | | **Upstream** |
| 420 | Reference probe 08839-L13359 | 2p13 | |
| 427 Ø | **SPRED1 probe** 13057-L14240 | | **Intron 5** |
| 436 Ø | **SPRED1 probe** 13058-L14241 | | **Intron 2** |
| 445 | Reference probe 12526-L13576 | 4q25 | |
| 454 | Reference probe 08579-L08580 | 17q23 | |

* New in version B3 (from lot B3-0818 onwards).
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of a single intron probe are unlikely to be related to the condition tested.

**Note:** The exon numbering used in this P295-B3 SPRED1 product description is the exon numbering from the RefSeq transcript NM_152594.2, which is identical to the NG_008980.1 sequence. The exon numbering and NM sequence used is from 09/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

## Table 2. *SPRED1* probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | *SPRED1* exon | Ligation site NM_152594.2 | Partial sequence (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *336-338 (Exon 1)* | | |
| 409 | 13056-L14239 | Upstream | 839 nt before exon 1 | GTGGCTCGACAA-CGATTTGGGAAA | 0.3 kb |
| 265 | 11013-L13179 | Upstream | 509 nt before exon 1 | CGGGCGTGAGGA-GCTGGCCCGCTG | 0.5 kb |
| 328 | 13053-L14236 | Exon 1 | 49-50 | GCCCCTCTCTTT-TTCCCTTTCCAC | 0.5 kb |
| 230 | 13050-L14233 | Exon 1 | 138 nt after exon 1 | AGATAGCTGATT-TCTTTAGCCATT | 8.4 kb |
| 185 Ø | 13048-L14231 | Intron 1 | 8515 nt after exon 1 | GGAATGTGGAAT-GTAATTCTTTTT | 31.7 kb |
| 274 Ø | 13051-L14234 | Intron 1 | 5928 nt before exon 2 | TGGAACCTAGAT-CTGACTTGTTAT | 6.0 kb |
| 214 | 11015-L28438 | Exon 2 | 416-417 | CGAGATGACTCA-AGTGGTGGATGG | 0.1 kb |
| 355 | 13054-L14237 | Exon 2 | 509-510 | TGTGCTGACTTT-TTTATCCGTGGA | 6.7 kb |
| 436 Ø | 13058-L14241 | Intron 2 | 6633 nt after exon 2 | CTGTCTGCCTTT-TTTCTCAAAGGT | 12.2 kb |
| 178 Ø | 13047-L14230 | Intron 2 | 3862 nt before exon 3 | GTATTTATGTGA-GTCAAAGAGCTA | 3.9 kb |
| 154 | 13044-L14227 | Exon 3 | 548-549 | TGTCAGGTGGTT-TTGGAATGTATG | 0.1 kb |
| 247 | 11016-L11685 | Exon 3 | 663-664 | CTGCTGATGCTA-GGGCTTTTGATA | 2.3 kb |
| 208 | 13049-L28437 | Exon 4 | 117 nt before exon 4 | TTGCCAGGCAGT-CCAGAAAGATCT | 0.2 kb |
| 196 | 11017-L11686 | Exon 4 | 17 nt after exon 4 | AATGGCTTGGAA-GGAATTTGTAAA | 10.6 kb |
| 160 Ø | 13045-L14228 | Intron 4 | 4307 nt before exon 5 | AAGACAGTGAAT-AGTAGGTATTGA | 4.3 kb |
| 172 | 13046-L14229 | Exon 5 | 776-777 | GAAGAGGATTCT-TCCAGTTCTCTA | 0.1 kb |
| 346 | 11018-L11687 | Exon 5 | 839-840 | AGTGAGCCTTAT-AGAAGCTCAAAT | 6.8 kb |
| 427 Ø | 13057-L14240 | Intron 5 | 2850 nt before exon 6 | TTCATCCTAGAG-GTAACTATTACC | 2.9 kb |
| 238 | 11019-L11688 | Exon 6 | 964-965 | CAGAAGTATGGA-ATACGTACAGCG | 0.1 kb |
| 292 | 13052-L14235 | Exon 6 | 84 nt after exon 6 | TAATTCTCCATA-TAGTTGAATTGT | 1.6 kb |
| 301 | 11020-L11689 | Exon 7 | 1174-1175 | TGATTCCAGTAT-TCAGTTTTCTAA | 0.4 kb |
| 402 | 11021-L11690 | Exon 7 | 1570-1571 | AGCTTTGTCTTT-CATTGTACCATG | 0.2 kb |
| 382 | 13055-L14238 | Exon 7 | 1749-1750 | AGCTTTTGGCAA-GCAATATGGAAT | |
| | | *stop codon* | *1668-1670 (Exon 7)* | | |

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of a single intron probe are unlikely to be related to the condition tested.

**Note:** The exon numbering used in this P295-B3 SPRED1 product description is the exon numbering from the RefSeq transcript NM_152594.2, which is identical to the NG_008980.1 sequence. The exon numbering and NM sequence used is from 09/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

## Related SALSA MLPA probemixes

| | |
|---|---|
| P044 NF2 | Contains probes for the *NF2* gene, involved in Neurofibromatosis type 2. |
| P081/P082 NF1 | Contains probes for the *NF1* gene, involved in Neurofibromatosis type 1. |
| P122 NF1 area | Contains probes for the 17q11.2 region, involved in Neurofibromatosis type 1. |
| P250 DiGeorge | Contains probes for the 22q11 region, used for primary screening of this region. |
| P258 SMARCB1 | Contains probes for the *SMARCB1* gene. Deletions and mutations in *SMARCB1* are associated with malignant rhabdoid tumours and schwannomatosis. |
| P455 LZTR1 | Contains probes for the *LZTR1* gene, involved in Neurofibromatosis type 3. |

## 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.

## Selected publications using SALSA MLPA Probemix P295 SPRED1


- Evans DG et al. (2016). Comprehensive RNA analysis of the NF1 gene in classically affected NF1 affected individuals meeting NIH criteria has high sensitivity and mutation negative testing is reassuring in isolated cases with pigmentary features only. *EBioMedicine*. 7:212-220.
- Palma Milla C et al. (2018). Neurofibromatosis type I: mutation spectrum of NF1 in spanish patients. *Ann Hum Genet*.
- Pasmant E et al. (2015). Neurofibromatosis type 1 molecular diagnosis: what can NGS do for you when you have a large gene with loss of function mutations? *Eur J Hum Genet*. 23(5):596-601.
- Pinna V et al. (2015). p. Arg1809Cys substitution in neurofibromin is associated with a distinctive NF1 phenotype without neurofibromas. *Eur J Hum Genet*. 23(8):1068-1071.
- Van Minkelen R et al. (2014). A clinical and genetic overview of 18 years neurofibromatosis type 1 molecular diagnostics in the Netherlands. *Clin Genet*. 85(4):318-327.
- Zhang J et al. (2016). Identification of a PTPN11 hot spot mutation in a child with atypical LEOPARD syndrome. *Mol Med Rep*. 14(3):2639-2643.


| **P295 Product history** | |
|---|---|
| Version | Modification |
| B3 | Two reference probes have been replaced. |
| B2 | One reference probe was added. |
| B1 | One reference probe has been removed and six have been replaced, furthermore the control fragments have been adjusted (QDX2). |
| A1 | First release. |

**Implemented changes in the product description**

*Version B3-01 – 03 October 2018 (01P)*
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Product description restructured and adapted to a new template.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

*Version 09 – 02 December 2016 (55)*
- Warning added below table 1 that an additional peak can appear at approximately 218 nt when the ligation reaction is at lower temperatures than recommended.

*Version 08 – 30 April 2015 (54)*
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Minor textual changes.

*Version 07 (49)*
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- Minor textual changes.

*Version 06 (48)*
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.


| **More information: www.mlpa.com; www.mlpa.eu** | |
|---|---|
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