# Product Description SALSA® MLPA® Probemix P211-B5 HSP region

To be used with the MLPA General Protocol.

**Version B5.** As compared to version B4, three reference probes have been replaced. For complete product history see page 7.

**Catalogue numbers:**
- **P211-025R:** SALSA MLPA Probemix P211 HSP region, 25 reactions.
- **P211-050R:** SALSA MLPA Probemix P211 HSP region, 50 reactions.
- **P211-100R:** SALSA MLPA Probemix P211 HSP region, 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 P211 HSP region is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SPAST* gene and other genes located in the 2p22.3 region and 15q11.2 region, which are associated with Hereditary spastic paraplegias (HSPs).

HSPs are a large and diverse group of genetically heterogeneous neurodegenerative disorders characterised by progressive lower limb/lower extremity spasticity and weakness. Defects in the *SPAST* gene (also known as *SPG4*) on chromosome 2p22 are identified as one of the causes for autosomal dominant pure HSP. The protein encoded by this gene is spastin which is involved in membrane shaping and modelling events.

This probemix contains probes for the first exon and intron and probes for exon 16 and 17 of the *SPAST* gene and the flanking regions of *SPAST* (2p22.3 region). This probemix furthermore contains probes for the *NIPA1* gene (also known as *SPG6*) and probes for the nearby genes of *NIPA1*, such as the *WHAMML1* and *HERC2P2* genes (15q11.2 region). Probes for these flanking genes of *NIPA1* have been included to distinguish *NIPA1* defects from larger deletions in the Prader-Willi/Angelman syndrome region. The database of genomic variants mentions several copy number changes in this genomic region that have been found in healthy individuals (see http://dgv.tcag.ca/dgv/app/home).

**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 *SPAST* exon numbering used in this P211-B5 HSP region product description is the exon numbering from the RefSeq transcript NM_014946.3, which is identical to the LRG_714 sequence. The exon numbering and NM_ sequence used have been retrieved on 06/2019. 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 P211-B5 HSP region contains 39 MLPA probes with amplification products between 128 and 462 nucleotides (nt). This includes 13 probes for the 2p22.3 region,

including probes for the *SPAST* gene. This probemix furthermore contains 13 probes for the 15q11.2 region, which encompasses the *NIPA1, NIPA2*, *WHAMML1*, and *HERC2P2* genes. In addition, 13 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, 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 reference 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 Hereditary spastic paraplegias. 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 reference probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

| Copy number status | Dosage quotient |
|---|---|
| Normal | 0.80 < DQ < 1.20 |
| Homozygous deletion | DQ = 0 |
| Heterozygous deletion | 0.40 < DQ < 0.65 |
| Heterozygous duplication | 1.30 < DQ < 1.65 |
| Heterozygous triplication/Homozygous duplication | 1.75 < DQ < 2.15 |
| Ambiguous copy number | All other values |

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

**Limitations of the procedure:**

- In most populations, the major cause of genetic defects in the *SPAST* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P211 HSP region.
- 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.

***SPAST* mutation database:** https://databases.lovd.nl/shared/genes/SPAST. 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 to MRC-Holland: info@mlpa.com.

## Table 1. SALSA MLPA Probemix P211-B5 HSP region

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | 2p22.3 region | 15q11.2 region |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 128 | Reference probe 00797-L00093 | 5q31 | | |
| 133 | **HERC2P2 probe** 09878-L22208 | | | **Exon 23** |
| 139 | **SPAST probe** 05659-L22209 | | **Exon 1** | |
| 148 * | Reference probe 04445-L03831 | 4q13 | | |
| 154 | **GOLGA6L2 probe** 09873-L10285 | | | **Exon 7** |
| 166 * | Reference probe 16058-L18232 | 9p21 | | |
| 173 | **SPAST probe** 07128-L06737 | | **Exon 17** | |
| 179 « | **WHAMML1 probe** 09875-L12280 | | | **Exon 2** |
| 184 | Reference probe 02312-L01803 | 19p13 | | |
| 190 | **SPAST probe** 08262-L08125 | | **Upstream** | |
| 196 Ø | **SPAST probe** 08263-L08126 | | **Intron 1** | |
| 202 | **NIPA2 probe** 07123-L05171 | | | **Exon 8** |
| 214 | **NIPA2 probe** 05767-L05718 | | | **Exon 4** |
| 220 | Reference probe 02947-L02379 | 7q31 | | |
| 226 Ø | **SPAST probe** 08264-L08127 | | **Intron 1** | |
| 235 | **SPAST probe** 05265-L04648 | | **Exon 1** | |
| 241 | **SPAST probe** 05658-L05111 | | **Exon 17** | |
| 256 | Reference probe 06007-L05432 | 2q36 | | |
| 265 | **NIPA1 probe** 07124-L05206 | | | **Exon 5** |
| 274 | Reference probe 05360-L04739 | 11p13 | | |
| 292 | Reference probe 08790-L11322 | 10q21 | | |
| 299 | **SPAST probe** 07127-L06736 | | **Exon 1** | |
| 314 | **GOLGA6L2 probe** 09874-L12277 | | | **Exon 8** |
| 320 « ¬ | **DPY30 probe** 07130-L12278 | | **Exon 5** | |
| 328 * | Reference probe 16583-L18726 | 12q24 | | |
| 337 ¬ | **SLC30A6 probe** 07132-L06741 | | **Exon 12** | |
| 346 | **TUBGCP5 probe** 01321-L12279 | | | **Exon 8** |
| 355 ¬ | **DPY30 probe** 07129-L06738 | | **Exon 2** | |
| 364 | Reference probe 10675-L11257 | 6p12 | | |
| 382 | **CYFIP1 probe** 01818-L01317 | | | **Exon 23** |
| 391 | **NIPA1 probe** 07125-L05755 | | | **Exon 3** |
| 400 | Reference probe 05343-L04730 | 1p21 | | |
| 409 | **MAGEL2 probe** 11155-L11839 | | | **Exon 1** |
| 418 | **NIPA2 probe** 05117-L04501 | | | **Exon 10** |
| 428 | **HERC2P2 probe** 09877-L11168 | | | **Exon 13** |
| 436 | **SPAST probe** 08261-L22286 | | **Upstream** | |
| 445 | **SPAST probe** 20720-L28598 | | **Exon 16** | |
| 454 | Reference probe 01051-L00620 | 8q21 | | |
| 462 | Reference probe 18948-L01619 | 13q13 | | |

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

* New in version B5.
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

## Table 2. P211-B5 probes arranged according to chromosomal location

Table 2a. 2p22.3 region

| Length (nt) | SALSA MLPA probe | *SPAST* exon[a] | Ligation site NM_014946.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 320 ¬ | 07130-L12278 | *DPY30* Exon 5 | NM_032574.3; 677-678. | TTTTGGATGTAT-AAGAACCTTCCG | 15.4 kb |
| 355 ¬ | 07129-L06738 | *DPY30* Exon 2 | NM_032574.3; 2 nt before exon 2. | TCTTGTTTTCCA-AGACTGGTATCC | 22.8 kb |
| | | | | | |
| | | *start codon* | *222-224 (Exon 1)* | | |
| 436 | 08261-L22286 | Upstream | 1349 nt before exon 1 | TGTGGAGATTCT-TGGAAGCTGGAA | 0.5 kb |
| 190 | 08262-L08125 | Upstream | 881 nt before exon 1 | TCATTAGAATAC-AGGGAGCAGAGA | 0.7 kb |
| 299 | 07127-L06736 | Exon 1 | 178 nt before exon 1 | AACTGCACATTG-GGAACTGTAGTT | 0.4 kb |
| 139 | 05659-L22209 | Exon 1 | 226-227 | GCTGTGAATGAA-TTCTCCGGGTGG | 0.1 kb |
| 235 | 05265-L04648 | Exon 1 | 378-379 | ACCTGTACTATT-TCTCCTACCCGC | 1.7 kb |
| 196 Ø | 08263-L08126 | Intron 1 | 1410 nt after exon 1 | CCTCTGCCAAAA-ACACCCACTTTT | 14.9 kb |
| 226 Ø | 08264-L08127 | Intron 1 | 6932 nt before exon 2 | GTCATAGAGTTA-AAAGAGAAATGT | 66.7 kb |
| 445 | 20720-L28598 | Exon 16 | 1921-1922 | ACTAAAACCAGA-ACAGGTGAAGAA | 7.2 kb |
| 241 | 05658-L05111 | Exon 17 | 2031-2032 | CTTTAGAAGCGT-ACATACGTTGGA | 1.9 kb |
| 173 | 07128-L06737 | Exon 17 | 3961-3962 | TAGCCATAAGGT-AAATCATGTCTC | 48.2 kb |
| | | *stop codon* | *2070-2072 (Exon 17)* | | |
| | | | | | |
| 337 ¬ | 07132-L06741 | *SLC30A6* Exon 12 | NM_017964.5; 741-742 | TGCTATAGCTAT-TGCCTTGATGAC | |

Table 2b. 15q11.2 region

| Length (nt) | SALSA MLPA probe | Gene/exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 346 | 01321-L12279 | *TUBGCP5* Exon 8 | NM_052903.6; 793-794. | CAGTGATCCATT-GTATGTTCCAGA | 122.3 kb |
| 382 | 01818-L01317 | *CYFIP1* Exon 23 | NM_014608.5; 2586-2587 | CTGTTGGAAATC-AACCGCATGACC | 36.4 kb |
| 418 | 05117-L04501 | *NIPA2* Exon 10 | NM_001184889.1; 2568-2569 | TGAGCATTCGAT-GGCCTTAGCACC | 8.9 kb |
| 202 | 07123-L05171 | *NIPA2* Exon 8 | NM_001184889.1; 1079-1080 | GTGGCCAACTTC-GCTGCGTATGCG | 13.3 kb |
| 214 | 05767-L05718 | *NIPA2* Exon 4 | NM_001184889.1; 614-615 | TCTGAGAATAGT-GAAGCAACTCAT | 21.4 kb |
| 265 | 07124-L05206 | *NIPA1* Exon 5 | NM_144599.4; 627-628 | GCTGGGCAGTTT-CACCGTGCCTTC | 11.7 kb |
| 391 | 07125-L05755 | *NIPA1* Exon 3 | NM_144599.4; 272-273 | GCCAGATTGGAA-ACTTCCTGGCTT | 144.3 kb |
| 179 # | 09875-L12280 | *WHAMML1* Exon 2 | NR_003521.1; 612-613 | TTCAAGGACACC-GAAAAGCCAACA | 106.4 kb |
| 133 # | 09878-L22208 | *HERC2P2* Exon 23 | NR_002824.3; 3506-3507 | GCCCAGTTAGAT-GACTACTTCCCT | 15.1 kb |
| 428 # | 09877-L11168 | *HERC2P2* Exon 13 | NR_002824.3; 1780-1781 | TACTGTTACAGA-TGTTCACAAATA | 358.1 kb |
| 314 # | 09874-L12277 | *GOLGA6L2* Exon 8 | NM_001304388.1; 2976-2977 | AAGAGAAAGATG-AAGATCATCAAT | 2.2 kb |
| 154 # | 09873-L10285 | *GOLGA6L2* Exon 7 | NM_001304388.1; 735-736 | AGGAGCTGAAGA-AGAAAAATGCCG | 202.1 kb |
| 409 | 11155-L11839 | *MAGEL2* Exon 1 | NM_019066.4; 3692-3693 | AGCAAGATGCTT-GTCCTGAGGTTT | |

**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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

## Related SALSA MLPA probemixes

| | |
|---|---|
| P165 HSP | Contains probes for the *SPG3A* and *SPAST* genes. |
| P213 HSP mix-2 | Contains probes for the *REEP1* and *SPG7* genes. |
| P306 SPG11 | Contains probes for the *SPG11* gene. |

## 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 P211 HSP region


- De Leva MF et al. (2010). Complex phenotype in an Italian family with a novel mutation in SPG3A. *J. Neurol.*, *257*(3), 328-331.
- Dong EL et al. (2018). Clinical spectrum and genetic landscape for hereditary spastic paraplegias in China. *Mol. Neurodegener. 13*(1), 36.
- Mészárosová AU et al. (2016). SPAST mutation spectrum and familial occurrence among Czech patients with pure hereditary spastic paraplegia. *J. Hum. Genet.*, *61*(10), 845.


| **P211 Product history** | |
|---|---|
| *Version* | *Modification* |
| B5 | Three reference probes have been replaced. |
| B4 | Four reference probes have been replaced, five flanking probes have been removed and one length has been adjusted. |
| B3 | Three reference probes have been replaced, one removed and the 88 and 96 nt control fragments have been replaced (QDX2). |
| B2 | Seven probes in the 15q11 region between the *NIPA* genes and *MKRN3* have been added. Two DD (DNA Denaturation) control fragments at 88 and 96 nt have been added. |
| A1 | First release. |

**Implemented changes in the product description**

*Version B5-01 — 02 July 2019 (02P)*
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *SLC30A6*, *TUBGCP5*, and *CYFIP1* genes updated according to new versions of the NM_ reference sequences.
- Warning added to Table 2b for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

*Version 16 - 27 October 2016 (55)*
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- New references added on page 1.
- Various minor textual and layout changes.

- Exon numbering of *NIPA1*, *CYPIP1* and *GOLGA6L2* genes adjusted.

*Version 15 (53)*

- Remark added under table 2 about two *HERC2P2* probes that seem to be frequently deleted.
- Figure 1 Capillary electrophoresis pattern generated with old MLPA buffer has been removed.
- "Peak area" replaced with "peak height".
- Updated link for "Database of Genomic Variants".
- Corrected text on data analysis.

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