Instructions for Use

SALSA® MLPA® Probemix P213 HSP mix-2

See also the MLPA General Protocol, and the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P213 HSP mix-2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P213 HSP mix-2**
Version	B3
Catalogue numbers	P213-025R (25 reactions) P213-050R (50 reactions) P213-100R (100 reactions)
Basic UDI-DI:	872021148P2135P
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening 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.

Regulatory Status	
IVD	EUROPE CE 2797
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in this Product Version:
B3 version compared to B2 version
Three reference probes have been replaced. One target probe has a small change in length but no change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P213 HSP mix-2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *REEP1* and *SPG7* genes, in order to confirm a potential cause for and clinical diagnosis of spastic paraplegia (SPG) type 31 and SPG type 7, respectively and for molecular genetic testing of at-risk family members. This assay is for use with genomic DNA isolated from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P213 HSP mix-2 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *REEP1* and *SPG7* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration (and) treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of spastic paraplegia.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80-1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*REEP1* and *SPG7*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>To determine the expected values in normal and affected populations, a study was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in section 5 of this product description can be used. Cut-off values for copy number determination were verified with P213 HSP mix-2 in 42 samples from healthy individuals with normal copy numbers and one sample with known CNVs. The expected FRs for the corresponding copy numbers were found in all samples tested.</td></tr>
<tr><td>Limit of Detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P213 HSP mix-2 on one sample without any aberration and expected results were obtained using both 50 and 250 ng of DNA and on artificial DNAs with CNVs using 50 ng of DNA and all expected results were obtained.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using P213 HSP mix-2 was performed to assess the potential for interference of endogenous and exogenous substances (at the concentrations shown in the table below) on genomic DNA in samples with known CNVs status.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 80/93 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 93/93 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 93/93 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 93/93 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 72/93 measurements</td></tr>
</table>
* Results are summarised for all probes across the sample tested. In addition, artificial DNAs have been tested and showed concordant results.

Endogenous (hemoglobin) and exogenous interfering substances (EDTA, heparin, salts (NaCl), and Fe^{3+}) were tested and shown to have mild, severe or no effects on P213 HSP mix-2 results. NaCl, Fe^{3+} and heparin did not interfere with copy number determination. EDTA led to ambiguous ratios and potential delayed results. Hemoglobin led to incorrect ratios and, consequently, to false results.

To minimise variability across 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.</td></tr>
<tr><td>Cross- reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences.

To assess the potential for binding of non-specific targets from human genomic DNA, in silico analysis of the probes used in the assay was performed using Human BLAT Search. All probes in P213 HSP mix-2 were found to be specific.

To support the above in silico data, quality tests on 42 wildtype samples, and a positive sample were carried out to determine whether probes are specific to their target sequence. All probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, one previously genotyped sample was tested using P213 HSP mix-2 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 465/465 data points, leading to a precision of 100%. In addition, artificial DNAs have been tested and showed concordant results (precision >99%).</td></tr>
<tr><td>Clinical validity*</td><td>REEP1: ~9.5% of Spastic paraplegia 31 (SPG31) is caused by deletions in REEP1 (Goizet et al. 2011).

SPG7: <2% of Spastic paraplegia 7 (SPG7) is caused by deletions in SPG7 (Arnoldi et al. 2008; Yoon et al. 2013).

*(Based on a 2018-2023 literature review)</td></tr>
</table>

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
2p11.2	*REEP1*	Exon 9 (8)	15.9 kb	260	07254-L08407	
2p11.2	*REEP1*	Exon 6 (7)	19.3 kb	220	07253-L08404	
2p11.2	*REEP1*	Exon 5 (6)	2.7 kb	328	07252-L06823	
2p11.2	*REEP1*	Exon 4 (5)	9.2 kb	172	20988-L29219	
2p11.2	*REEP1*	Exon 3 (4)	18.1 kb	400	07250-L06821	
2p11.2	*REEP1*	Intron 2 (Exon 3)	0.2 kb	310	07249-L21919	Ø
2p11.2	*REEP1*	Exon 2 (3)	55.3 kb	142	07248-L06819	
2p11.2	*REEP1*	Exon 1 (2)	0.5 kb	202	07246-L06817	
2p11.2	*REEP1*	Upstream (Exon 1)	0.1 kb	238	17747-L21893	Ø
2p11.2	*REEP1*	Upstream (Exon 1)		382	07245-L21921	Ø
16q24.3	*SPG7*	Upstream	0.9 kb	268	07256-L08408	Ø
16q24.3	*SPG7*	Exon 1	0.5 kb	214	07257-L21923	
16q24.3	*SPG7*	Intron 1	1.7 kb	427	07258-L06829	Ø
16q24.3	*SPG7*	Exon 2	2.4 kb	160	17745-L21891	
16q24.3	*SPG7*	Exon 3	11.1 kb	283	17749-L21895	
16q24.3	*SPG7*	Exon 4	2.2 kb	245	07261-L08406	
16q24.3	*SPG7*	Exon 5	3.2 kb	190	07262-L21916	
16q24.3	*SPG7*	Exon 6	1.2 kb	208	07263-L06834	
16q24.3	*SPG7*	Exon 7	1.1 kb	298	21471-L21918	
16q24.3	*SPG7*	Exon 8	0.6 kb	148	07265-L06836	
16q24.3	*SPG7*	Exon 9	4.5 kb	232	07266-L06837	
16q24.3	*SPG7*	Intron 9	0.4 kb	177	17746-L21892	Ø, ¥
16q24.3	*SPG7*	Intron 9	7.2 kb	391	17750-L21896	Ø, ¥
16q24.3	*SPG7*	Exon 10	1.9 kb	337	07267-L06838	
16q24.3	*SPG7*	Exon 11	1.3 kb	166	07268-L06839	
16q24.3	*SPG7*	Exon 12	2.5 kb	408	17751-L21897	
16q24.3	*SPG7*	Exon 13	2.5 kb	276	17902-L22475	
16q24.3	*SPG7*	Exon 14	0.9 kb	196	07271-L21917	
16q24.3	*SPG7*	Exon 15	0.7 kb	319	07272-L06843	
16q24.3	*SPG7*	Exon 16	2.5 kb	363	07273-L21920	
16q24.3	*SPG7*	Exon 17		226	07274-L08405	
2q	Reference			154	04531-L05030	
3q	Reference			133	16316-L20697	
5p	Reference			346	04835-L04219	
5q	Reference			128	00797-L00093	
6p	Reference			373	10693-L11275	
7q	Reference			417	18456-L23632	
11p	Reference			292	08936-L09031	
12q	Reference			436	04279-L23590	
14q	Reference			184	19450-L25864	
20p	Reference			355	05991-L05416	
21q	Reference			253	06236-L01311	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *REEP1* and *SPG7* exon numbers are derived from MANE project and are based on MANE Select transcripts. For more information, see the probe sequences document available on the product page at www.mrcholland.com. The exon numbering from the B3-02 version of this Product Description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

Ø These probes target sequences outside of the known coding region of the MANE Select transcript.

¥ These probes detect an alternative exon 10 which is only present in transcript variant 2 NM_199367.3.

Probemix-specific precautions

1. This product 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).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-

probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. No probes for *REEP1* exons 7 and 8 are present.
2. The clinical significance of duplications in *REEP1* and *SPG7* is not yet clearly established.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Arnoldi A et al. (2008). A clinical, genetic, and biochemical characterization of SPG7 mutations in a large cohort of patients with hereditary spastic paraplegia. *Human mutation*. 29:522-531.
2. Goizet C et al. (2011). REEP1 mutations in SPG31: frequency, mutational spectrum, and potential association with mitochondrial morpho-functional dysfunction. *Human mutation*. 32:1118-1127.
3. Yoon G et al. (2013). Autosomal recessive hereditary spastic paraplegia—clinical and genetic characteristics of a well-defined cohort. *Neurogenetics*. 14:181-188

Implemented changes in the product description

Version B3-04 – 9 December 2025 (03S)
- Two reference probes 05991-L05416 and 06236-L01311 that were omitted in version B3-03 were added back to table Content – Probe Details Sorted by Chromosomal Position.
- A warning was added for *SPG7* probes 17746-L21892 and 17750-L21896 detecting exon 10 which is only present in an alternative transcript variant.
- A warning related to the *REEP1* probe 07246-L06817, which detects exon 2 in an alternative transcript variant and exon 1 in MANE Select, was removed in version B3-03.
- The probe warning regarding probes which target sequences outside of the known coding region of the MANE Select transcript was edited to remove the statement that copy number alterations of only those probes are of unknown clinical significance.
- Remark added to Performance Characteristics table that artificial DNAs have been tested in accuracy studies.

Version B3-03 – 21 March 2025 (03S)
- The product description has been updated to a new template.
- The intended purpose has been updated removing the duplications for *REEP1* and *SPG7* genes from the IVD claim and specifying assay is manual.
- Exon numbering for all probes targeting the *REEP1* gene updated.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning for probes located outside of coding region added to probes: 07245-L21921, 17747-L21893, 07249-L21919, 07256-L08408, 07258-L06829, 17746-L21892 and 17750-L21896.
- Warning for probes located in or near a GC-rich region (salt contamination) removed for probes: 07246-L06817, 17747-L21893 and 07245-L21921.
- Probemix is now IVDR-certified.