Product Description
SALSA® MLPA® Probemix P136-B4 Gitelman Syndrome

To be used with the MLPA General Protocol.

Version B4
As compared to version B3, one probe length has been adjusted, one reference probe has been replaced and three reference probes have been removed. For complete product history see page 7.

Catalogue numbers:
- **P136-025R:** SALSA MLPA Probemix P136 Gitelman Syndrome, 25 reactions.
- **P136-050R:** SALSA MLPA Probemix P136 Gitelman Syndrome, 50 reactions.
- **P136-100R:** SALSA MLPA Probemix P136 Gitelman Syndrome, 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.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, 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.

General information
The SALSA MLPA Probemix P136 Gitelman Syndrome is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SLC12A3* gene, which is associated with Gitelman syndrome.

The Gitelman variant of Bartter Syndrome (OMIM 263800) is an autosomal recessive disorder characterised by hypokalemic alkalosis combined with hypomagnesemia, low urinary calcium, and increased renin activity. It is mainly caused by defects in the *SLC12A3* gene on chromosome 16.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1338/.

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 *SLC12A3* exon numbering used in this P136-B4 Gitelman Syndrome product description is the exon numbering from the RefSeq transcript NM_000339.3. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P136-B4 Gitelman Syndrome contains 36 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 26 probes for the *SLC12A3* gene, one probe for each exon, with the exception of exon 6. In addition, ten 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).

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 the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.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 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 different unrelated individuals who are from families without a history of Gitelman Syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore samples should

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.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 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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

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

- 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 or flanking 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

- In most populations, the major cause of genetic defects in the *SLC12A3* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P136 Gitelman Syndrome.
- 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.

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.

LOVD mutation database

https://databases.lovd.nl/shared/genes/SLC12A3. 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 SNVs and unusual results (e.g., a duplication of *SLC12A3* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P136-B4 Gitelman Syndrome

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*SLC12A3*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q	
136	**SLC12A3 probe** 15900-L17372		**Intron 6**
142	**SLC12A3 probe** 05541-L03937		**Exon 1**
148	**SLC12A3 probe** 15518-L17992		**Exon 10**
155	**SLC12A3 probe** 04566-L03955		**Exon 19**
160	Reference probe 19366-L25759	3p	
166	**SLC12A3 probe** 04549-L03938		**Exon 2**
172	**SLC12A3 probe** 04558-L03947		**Exon 11**
178	**SLC12A3 probe** 04567-L03956		**Exon 20**
190	Reference probe 06378-L05844	6p	
196	**SLC12A3 probe** 15519-L17374		**Exon 3**
205	**SLC12A3 probe** 15520-L17993		**Exon 12**
211	**SLC12A3 probe** 15521-L17376		**Exon 26**
220 *	Reference probe 21710-L30368	15q	
229	**SLC12A3 probe** 15522-L17377		**Exon 4**
238	**SLC12A3 probe** 04560-L03949		**Exon 13**
247	**SLC12A3 probe** 04569-L03958		**Exon 22**
265	Reference probe 14759-L16456	11q	
274	**SLC12A3 probe** 05540-L03941		**Exon 5**
283 ¥	**SLC12A3 probe** 23095-L32593		**Exon 14**
292	**SLC12A3 probe** 04570-L03959		**Exon 23**
301	Reference probe 17452-L21208	12p	
310	**SLC12A3 probe** 05207-L03957		**Exon 21**
319	**SLC12A3 probe** 04562-L03951		**Exon 15**
328	**SLC12A3 probe** 04571-L17061		**Exon 24**
346	Reference probe 09073-L22926	19p	
355	**SLC12A3 probe** 15523-L17378		**Exon 7**
364	**SLC12A3 probe** 15524-L17379		**Exon 16**
373	**SLC12A3 probe** 15525-L17380		**Exon 25**
382	Reference probe 02770-L02160	9q	
391	**SLC12A3 probe** 15526-L17381		**Exon 8**
400	**SLC12A3 probe** 15527-L17382		**Exon 17**
409	Reference probe 10485-L11038	2p	
436	**SLC12A3 probe** 15528-L17383		**Exon 9**
445	**SLC12A3 probe** 04565-L03954		**Exon 18**
454	Reference probe 08579-L08580	17q	

[a] See section Exon numbering on page 1 for more information.

* New in version B4.
¥ Changed in version B4. Minor alteration, no change in sequence detected.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. SLC12A3 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*SLC12A3* exon[a]	Ligation site NM_000339.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*30-32 (Exon 1)*		
142	05541-L03937	Exon 1	140-141	CCAGCTGCCTAT-GACAGCAGCCAC	1.8 kb
166	04549-L03938	Exon 2	344-345	GCCCTGGCCTTT-GACAGCCGGCCC	1.0 kb
196	15519-L17374	Exon 3	146 nt before exon 3, reverse	GTAGGTTCCGAG-TCTGGTTCTATG	1.6 kb
229	15522-L17377	Exon 4	567-568	TGCTGTCGGTCA-CGGTGACCTCCA	0.4 kb
274	05540-L03941	Exon 5	685-686	TGGGGGCTCCAT-CGGCCTCATTTT	1.1 kb
136	15900-L17372	Intron 6	538 nt after exon 6	CTGGTCCACCAT-ACCCACATCCCC	1.1 kb
355	15523-L17378	Exon 7	910-911	CCTTGTCATCAT-GGTCTCCTTTGC	0.3 kb
391	15526-L17381	Exon 8	998-997, reverse	TGGACAAAAATG-TCCGCTGGAAAA	5.5 kb
436	15528-L17383	Exon 9	1186-1187	GACCATTTCCTA-CCTGGCCATCTC	1.0 kb
148	15518-L17992	Exon 10	1324-1325	CGAGTGCACCCA-GCAGCACAGCTG	0.5 kb
172	04558-L03947	Exon 11	1468-1469	TGCCAAAGTCTT-CCAGGTGAGGCC	0.5 kb
205	15520-L17993	Exon 12	1496-1497	GACCAGCTGTAC-CCACTGATCGGC	2.3 kb
238	04560-L03949	Exon 13	1633-1634	CATTTCCAACTT-CTTCCTCTGCTC	1.7 kb
283	23095-L32593	Exon 14	1774-1775	GGTCATCATGTT-CCTCCTCACCTG	1.2 kb
319	04562-L03951	Exon 15	1876-1877	GGGCTCCTCGGT-ACAGGCTGGCTC	1.2 kb
364	15524-L17379	Exon 16	2035-2036	CACCCGGAACCT-CAGCCTGATGAT	0.6 kb
400	15527-L17382	Exon 17	2142-2143	ACAAGAGGAAGA-TCAAGGCCTTCT	0.9 kb
445	04565-L03954	Exon 18	2240-2241	AAGCCCAACATT-CTGGTGGTTGGG	2.4 kb
155	04566-L03955	Exon 19	2354-2355	TGTGTCATGAGG-ATGCGGGAGGGA	1.8 kb
178	04567-L03956	Exon 20	1 nt before exon 20	TCTTCTTTTGCA-GTTAACCCCGTG	0.9 kb
310	05207-L03957	Exon 21	2526-2527	CCATCTTCCAGT-CGGAGCAGGGCA	1.5 kb
247	04569-L03958	Exon 22	6 nt before exon 22	GGGGACTCTCCT-TGCCAGGCCTCA	5.0 kb
292	04570-L03959	Exon 23	2719-2720	CAAGTTCCGACT-GGGATTCCATGA	2.9 kb
328	04571-L17061	Exon 24	2878-2879	CCCCTGGAAGAT-CTCAGATGAGGA	1.9 kb
373	15525-L17380	Exon 25	2936-2937	GTGAGGCTGAAT-GAGATTGTGCTG	9.0 kb
211	15521-L17376	Exon 26	3110-3111	GTGCTCACCTTT-TACTGCCAGTAA	
		stop codon	*3120-3122 (Exon 26)*		

[a] See section Exon numbering on page 1 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P177 CASR	Contains probes for the *CASR* gene.
P266 CLCNKB	Contains probes for the *CLCNKB* gene involved in Bartter syndrome.

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 P136 Gitelman Syndrome

- Börklü-Yücel E et al. (2020). Clinical exome sequencing in neuromuscular diseases: an experience from Turkey. *Neurol Sci*, 41(8), 2157-2164.
- Fanis P et al. (2019). A novel heterozygous duplication of the SLC12A3 gene in two Gitelman syndrome pedigrees: indicating a founder effect. *J Genet*, 98(1), 5.
- Matsunoshita N et al. (2016). Differential diagnosis of Bartter syndrome, Gitelman syndrome, and pseudo-Bartter/Gitelman syndrome based on clinical characteristics. *Genet Med*. 18.2: 180.
- Ohkubo K et al. (2014). A novel mutation of CLCNKB in a Japanese patient of Gitelman-like phenotype with diuretic insensitivity to thiazide administration. *Meta gene* 2: 342-348.
- Sahbani D et al. (2020). Functional Study of Novel Bartter's Syndrome Mutations in ClC-Kb and Rescue by the Accessory Subunit Barttin Toward Personalized Medicine. *Front Pharmacol* 11: 327.
- Subasinghe CJ et al. (2017). Novel mutation in the SLC12A3 gene in a Sri Lankan family with Gitelman syndrome & coexistent diabetes: a case report. *BMC nephrol*, 18(1), 1-5

P136 product history	
Version	*Modification*
B4	One probe length has been adjusted, one reference probe has been replaced and three reference probes have been removed.
B3	Six reference probes have been replaced and three reference probes have been removed.
B2	The control fragments have been adjusted (QDX2).
B1	Eleven SLC12A3 probes have been replaced and one extra SLC12A3 probe has been added. In addition, four control fragments at 88, 96 (D-fragments) and 100, 105 nt (X, Y chromosome) have been included.
A1	First release.

Implemented changes in the product description

Version B4-01 – 20 May 2021 (04P)
- 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 *SLC12A3* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 12 – 21 September 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- New references added on page 1.
- Various minor textual changes on pages 1 and 2.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 11 (53)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Minor textual changes.

Version 10 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 09 (46)
- Various minor textual changes.
- Remark on RefSeqGene standard and transcript variant added below Table 2.

Version 08 (46)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Various minor textual changes on page 1.

More information: www.mrcholland.com; www.mrcholland.eu	
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