Product Description SALSA® MLPA® Probemix P234-A4 GATA3 - GATA4

To be used with the MLPA General Protocol.

Version A4. As compared to version A3, four reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P234-025R:** SALSA MLPA Probemix P234 GATA3 - GATA4, 25 reactions.
- **P234-050R:** SALSA MLPA Probemix P234 GATA3 - GATA4, 50 reactions.
- **P234-100R:** SALSA MLPA Probemix P234 GATA3 - GATA4, 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 P234 GATA3 - GATA4 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GATA3* and *GATA4* genes.

The *GATA4* gene encodes a member of the GATA family of zinc-finger transcription factors. Members of this family recognise the GATA motif, which is present in the promoters of many genes. This protein is thought to regulate genes involved in embryogenesis and in myocardial differentiation and function. Mutations in the *GATA4* gene have been associated with cardiac septal defects (OMIM#600576).

Microdeletions of chromosome 10p14, affecting among others the *GATA3* gene, result in a DiGeorge-like phenotype that includes hypoparathyroidism, heart defects, immune deficiency, deafness, and renal malformations. One region that contributes to this complex phenotype is that for the syndrome of hypoparathyroidism, sensorineural deafness, and renal insufficiency (HDR). The HDR dysplasia syndrome is an autosomal dominant disorder caused by mutations or intragenic deletions of the dual zinc finger transcription factor, *GATA3* (OMIM#131320).

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 *GATA3* and *GATA4* exon numbering used in this P234-A4 GATA3 - GATA4 product description is the exon numbering from the RefSeq transcripts NM_001002295.2 and NM_002052.5, which are identical to the NG_015859.1 and NG_008177.2 sequences respectively. The exon numbering and NM_ sequence used have been retrieved on 06/2020. 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 P234-A4 GATA3 - GATA4 contains 28 MLPA probes with amplification products between 130 and 373 nucleotides (nt). This includes five probes for the *GATA3* gene, one probe for each exon except for exon 2, and one probe downstream of the gene. Furthermore, eight

probes for the *GATA4* gene are included, one probe for each of the exons and two probes for exon 7, and three probes upstream (including *BLK* gene) and one probe downstream of the gene (*FDFT1* gene). 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.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, 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.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 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 heart defects. 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 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 *GATA3* and *GATA4* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P234 GATA3 - GATA4.
- 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.

***GATA3* and *GATA4* mutation database:** https://databases.lovd.nl/shared/genes/GATA3 and https://databases.lovd.nl/shared/genes/GATA4. 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 *GATA4* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P234-A4 GATA3 - GATA4

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*GATA3*	*GATA4*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L19287	5q31		
136	**GATA3 probe** 07632-L07317		**Exon 1**	
142	**GATA4 probe** 07641-L07326			**Exon 5**
154 *	Reference probe 10096-L10520	8q22		
165	**GATA3 probe** 07635-L07320		**Exon 5**	
175 ¬	**BLK probe** 03655-L03068			**Upstream**
180 ¬	**FDFT1 probe** 21103-L02483			**Downstream**
190 *	Reference probe 08129-L25574	11p15		
202	**GATA4 probe** 07643-L07328			**Exon 7**
211	**GATA3 probe** 07634-L07319		**Exon 4**	
220	Reference probe 05282-L04663	14q22		
229 «	**GATA4 probe** 07697-L07414			**Upstream**
238	**GATA4 probe** 07642-L07327			**Exon 6**
244 *	Reference probe 16266-L19130	20q11		
256	Reference probe 02871-L02338	1p22		
265	**GATA3 probe** 01225-L00776		**Exon 3**	
274	**GATA4 probe** 01226-L03844			**Exon 7**
283 *	Reference probe 10732-L11314	6p12		
292	Reference probe 05581-L05456	9q22		
301	**GATA3 probe** 07636-L07321		**Exon 6**	
308	**GATA4 probe** 21104-L07323			**Exon 2**
319	**GATA4 probe** 11619-L11083			**Exon 1**
330	Reference probe 17586-L21885	7q11		
337	**GATA4 probe** 07639-L07324			**Exon 3**
346 ¬	**CELF2 probe** 01232-L00780		**Downstream**	
355	**GATA4 probe** 07640-L07325			**Exon 4**
362 «	**GATA4 probe** 07696-L07413			**Upstream**
373	Reference probe 02560-L02023	3q23		

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

* New in version A4.
« 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.

Table 2. P234-A4 probes arranged according to chromosomal location

Table 2a. *GATA3*

Length (nt)	SALSA MLPA probe	*GATA3* exon[a]	Ligation site NM_001002295.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*574-576 (Exon 2)*		
136	07632-L07317	Exon 1	146-147	GAGCAACGCAAT-CTGACCGAGCAG	3.8 kb
	No probe	Exon 2			
265	01225-L00776	Exon 3	1128-1129	GAGTGCCTCAAG-TACCAGGTGCCC	5.4 kb
211	07634-L07319	Exon 4	1386-1387	GGGGCAACCTCG-ACCCCACTGTGG	5.6 kb
165	07635-L07320	Exon 5	1617-1618	TACTACAAGCTT-CACAATGTAAGT	4.3 kb
301	07636-L07321	Exon 6	1749-1750	AACAGCTCGTTT-AACCCGGCCGCC	2433.2 kb
		stop codon	*1906-1908 (Exon 6)*		
346 ¬	01232-L00780	*CELF2*	2433 kb downstream	TGTAGACCACAT-GATGGAGATTTG	

Table 2b. *GATA4*

Length (nt)	SALSA MLPA probe	*GATA4* exon[a]	Ligation site NM_002052.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
175 ¬	03655-L03068	*BLK*	140 kb upstream	AAGCCCCAGTAA-GGTGTTCAGGAC	131.5 kb
362 «	07696-L07413	Upstream	8.4 kb upstream of exon 1	CATGCTCAAGAT-AGGCACTGGAGC	1.5 kb
229 «	07697-L07414	Upstream	6.9 kb upstream of exon 1	GAGGTTCTTCTT-TAAAATCCATTC	7.0 kb
		start codon	*561-563 (Exon 2)*		
319	11619-L11083	Exon 1	2 nt after exon 1	GGCCCAGCAGGT-AGGGCTTTTTTC	3.8 kb
308	21104-L07323	Exon 2	394-395	TGCTGGATTTAA-TACGTATATATT	40.8 kb
337	07639-L07324	Exon 3	1180-1181	CTCAGTAGATAT-GTTTGACGACTT	1.3 kb
355	07640-L07325	Exon 4	1462-1463	CTACATGAAGCT-CCACGGGGTACG	4.9 kb
142	07641-L07326	Exon 5	1538-1539	AAGAACCTGAAT-AAATCTAAGACA	1.9 kb
238	07642-L07327	Exon 6	1615-1616	CAACTCCAGCAA-CGCCACCACCAG	1.4 kb
202	07643-L07328	Exon 7	1791-1792	CACAAGGCTATG-CGTCTCCCGTCA	0.3 kb
274	01226-L03844	Exon 7	2068-2069	TGGATTTTCTCA-GATGCCTTTACA	50.2 kb
		stop codon	*1887-1889 (Exon 7)*		
180 ¬	21103-L02483	*FDFT1*	50 kb downstream	GCCTGAAAACTT-GCTACAAGTATC	

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.

« 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.

Related SALSA MLPA probemixes

P250 DiGeorge Contains probes for the 22q11 DiGeorge region.
P311 CHD Contains more probes for congenital heart disease.

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 P234 GATA3 - GATA4

- Guida V et al. (2010). Multiplex ligation-dependent probe amplification analysis of GATA4 gene copy number variations in patients with isolated congenital heart disease. *Dis Markers*, 28(5), 287-292.
- Harrison SM et al. (2014). DNA copy number variations in patients with 46, XY disorders of sex development. *J Urol*, 192(6), 1801-1806.
- Joseph AD et al. (2019). Hypoparathyroidism, Sensorineural deafness and renal disease (Barakat syndrome) caused by a reduced gene dosage in GATA3: a case report and review of literature. *BMC Endocr Disord*, 19(1), 111.
- Nagy O et al. (2019). Copy number variants detection by microarray and multiplex ligation-dependent probe amplification in congenital heart diseases. *J Biotechnol*, 299, 86-95.

P234 Product history	
Version	*Modification*
A4	Four reference probes have been replaced.
A3	Two lengths have been adjusted.
A2	Three reference probes have been replaced and the control fragments have been adjusted (QDX2).
A1	First release.

Implemented changes in the product description

Version A4-01 — 13 July 2020 (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 both *GATA3* and *GATA4* genes updated according to new version of the NM_ reference sequences.

Version 12 – 06 April 2018 (55)
- Various minor textual changes.

Version 11 – 19 September 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and 2, new picture included).
- Product name adjusted from GATA4 to GATA3 – GATA4.
- Name of DKFZp566L0824 probe adjusted to CELF2.
- Updated link to "Database of Genomic Variants".

Version 10 (49)
- Electropherogram picture of the old buffer (introduced Dec. 2012) removed.

Version 09 (49)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).

Version 08 (48)
- Warning added in Table 1, 130 nt probe 02269-L01761.

Version 07 (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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