Instructions for Use
SALSA® MLPA® Probemix P159 GLA

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

Visit the SALSA® MLPA® Probemix P159 GLA product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P159 GLA**
Version	A5
Catalogue numbers	P159-025R (25 reactions) P159-050R (50 reactions) P159-100R (100 reactions)
Basic UDI-DI	872021148P1596A
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

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

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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.

More Information: www.mrcholland.com	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

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 or country in which the user and/or the patient is located.

Changes in this Product Version
As compared to version A4, the length of the Y control fragment has been elongated from 118 nt to 121 nt.

1. Intended Purpose

The SALSA MLPA Probemix P159 GLA is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *GLA* gene in genomic DNA isolated from human peripheral whole blood specimens. P159 GLA is intended to confirm a potential cause for and clinical diagnosis of Fabry disease and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P159 GLA 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 *GLA* gene 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.

[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 Fabry disease.	
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	
	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
105-121	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 X Probes (*GLA*)

Final Ratio (FR)	Copy Number Female	Copy Number Male	Description
0	0	0	**Female:** Homozygous deletion **Male**: Deletion
0.40 – 0.65	1	-	**Female:** Heterozygous deletion
0.80 – 1.20	**2**	**1**	**Normal**
1.30 – 1.65	3	-	**Female:** Heterozygous duplication
1.75 – 2.15	4	2	**Female**: Homozygous duplication or Heterozygous triplication **Male:** Duplication
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

Study	Description
Expected values for copy numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 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 the product description can be used. Cut-off values for copy number determination were verified with SALSA® MLPA® Probemix P159 GLA in 52 samples from healthy individuals (male and female) with normal copy numbers and two samples (female) with a previously genotyped large deletion on the X-chromosome, including the *GLA* gene. The expected FRs for the corresponding copy number were found in all samples tested.
Limit of detection	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 SALSA® MLPA® Probemix P159 GLA on one sample with a large deletion on the X-chromosome, which includes the *GLA* gene, and on one sample with a normal copy number for the *GLA* gene and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	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 SALSA® MLPA® Probemix P159 GLA was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNV status. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 44/48 measurements

	<table><tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 48/48 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 48/48 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 48/48 measurements</td></tr><tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 43/48 measurements</td></tr></table>* Results are summarised for 8 *GLA* probes across two samples tested in duplicate. Hemoglobin and EDTA had the largest effect on copy number determination, as FRs within an ambiguous range were found in both samples. DNA extraction methods remove hemoglobin and EDTA. Therefore, it is only when hemoglobin and/or EDTA are present in excess that ambiguous results for these probes can be found and would at most lead to a delayed result, as the assay may have to be repeated. No false positives or negatives would be obtained. Additionally, Coffalyser.Net issues warnings for the samples in which hemoglobin showed an effect, as well as lowered quality scores. None of the other interfering substances affected the FRs in these samples. 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.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, twenty-five previously genotyped sample was tested using SALSA® MLPA® Probemix P159 GLA and found to have the expected results in 224/224 measurements. 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 240/240 measurements, leading to a precision of 100%.
Clinical validity*	*GLA*: large deletions and duplications in the *GLA* gene explain up to 2.9-6.7% of FD cases (Higuchi et al. 2016, Perretta et al. 2018, Schirinzi et al. 2008, Yoshimitsu et al. 2011). *Based on a 2008-2025 literature review

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
Xq22.1	Flanking probe 6		21.7 kb	196	06926-L06506	¬
Xq22.1	Flanking probe 5		24.2 kb	337	06911-L06491	¬
Xq22.1	Flanking probe 4		2.1 kb	283	05183-L04555	¬
Xq22.1	*GLA*	Exon 7	0.5 kb	238	05160-L04564	
Xq22.1	*GLA*	Exon 6	0.5 kb	217	05159-L28097	
Xq22.1	*GLA*	Exon 5	1.8 kb	185	05158-L08283	
Xq22.1	*GLA*	Exon 4	1.0 kb	319	06500-L04561	
Xq22.1	*GLA*	Exon 3	2.1 kb	256	05156-L04560	
Xq22.1	*GLA*	Exon 2	0.1 kb	222	05178-L28098	
Xq22.1	*GLA*	Exon 2	3.8 kb	206	05154-L28096	
Xq22.1	*GLA*	Exon 1	0.5 kb	178	05153-L04557	
Xq22.1	Flanking probe 3 (*HNRNPH2*)		2.4 **M**b	265	05181-L04553	¬ ∫
Xq22.1	Flanking probe 2		4.8 **M**b	154	01274-L01640	¬
Xq22.1	Flanking probe 1			291	05856-L05256	¬
Xp	Reference			247	03650-L03063	
Xp	Reference			298	06476-L26217	
Xp	Reference			229	02922-L04201	
Xp	Reference			146	01394-L01042	
Xp	Reference			161	16675-L20191	
Xp	Reference			190	07653-L14466	
Xq	Reference			328	12605-L13689	
Xq	Reference			355	13524-L14330	
Xq	Reference			275	04124-L03481	
Xq	Reference			166	02927-L03721	
Xq	Reference			310	03493-L02870	

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 *GLA* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

¬ These probes are flanking probes, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.

∫ This flanking probe has been reported to be part of a deletion that also comprises *GLA* exons 1 and 2 (Schirinzi et al. 2008).

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

1. Deletion or duplication of only the flanking probes are not expected to be the cause of Fabry disease. These probes have only been included to delineate the extent of large deletions and duplications.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Higuchi T et al. (2016). Identification of Cryptic Novel alpha-Galactosidase A Gene Mutations: Abnormal mRNA Splicing and Large Deletions. *JIMD Rep.* 30:63-72.
2. Perretta F et al. (2018). Major Organic Involvement in Women with Fabry Disease in Argentina. *The Scientific World Journal.* 2018.
3. Schirinzi A et al. (2008). Identification of GLA gene deletions in Fabry patients by Multiplex Ligation-dependent Probe Amplification (MLPA). *Mol Genet Metab.* 94:382-385.
4. Yoshimitsu M et al. (2011). Identification of novel mutations in the alpha-galactosidase A gene in patients with Fabry disease: pitfalls of mutation analyses in patients with low alpha-galactosidase A activity. *J Cardiol.* 57:345-353.

Implemented changes in the product description
Version A5-06 – 26 June 2025 (03S) - The product description has been adapted to a new template. - The intended purpose was updated to include that the assay is manual. - SNV rs104894841 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section 7. Precautions and Warnings. - A limitation was added to Section 8. Limitations, clarifying the purpose of flanking probes. - Probemix is now IVDR certified.