Product Description SALSA® MLPA® Probemix P156-B3 GALT

To be used with the MLPA General Protocol.

Version B3. As compared to version B2, one reference probe has been removed and two reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P156-025R:** SALSA MLPA Probemix P156 GALT, 25 reactions.
- **P156-050R:** SALSA MLPA Probemix P156 GALT, 50 reactions.
- **P156-100R:** SALSA MLPA Probemix P156 GALT, 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 P156 GALT is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GALT* gene, which is associated with Classic Galactosemia and Clinical Variant Galactosemia.

Classic galactosemia and clinical variant galactosemia are disorders of the galactose metabolism inherited in an autosomal recessive manner. These disorders are caused by defects of the *GALT* gene, encoding galactose-1-phosphate uridylyltransferase. Failure to thrive is the most common initial clinical symptom of galactosemia. The cardinal features of these disorders are hepatomegaly, cataracts, and intellectual disability. After exclusion of galactose from the diet, these signs and symptoms normalise. Classic galactosemia is estimated to occur 1 in 30,000 to 60,000 newborns, while the prevalence for the clinical variant galactosemia is unknown. Some cases of clinical variant galactosemia might be missed during the newborn screening, since breath testing is normal and hypergalactosemia is not as marked as in the classic galactosemia.

The *GALT* gene has 11 exons, spans ~4.0 kb of genomic DNA, and is located on chromosome 9p13.3, about 35 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1518/.

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 *GALT* exon numbering used in this P156-B3 GALT product description is the exon numbering from the RefSeqGene NG_009029.2. The exon numbering and NG_ sequence used have been retrieved on 07/2019. 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 P156-B3 GALT contains 22 MLPA probes with amplification products between 140 and 337 nucleotides (nt). This includes 11 probes covering all the exons of the *GALT* gene, one probe for the *DNAI1* gene located upstream of *GALT* gene, and one probe for the downstream *IL11RA* gene. In addition, nine 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 galactosemia. 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. Sample ID numbers NA01741 and NA05067 from the Coriell Institute have been tested with this P156 probemix at MRC-Holland and can be used as positive control samples to detect an homozygous deletion of the *GALT* gene (the flanking genes are not affected), and an heterozygous duplication of the *GALT* gene, and the flanking genes *DNAI1* and *IL11RA*, respectively. 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.

P156 specific note:

- Two common mutations, Q188R and K285N, account for 70% of the alleles causing galactosemia in Caucasians. The S135L allele accounts for 62% of the alleles causing galactosemia in the African American population (Elsas and Lai 1998). Specific probes for these mutations are not included. No probes specific for these mutations are included.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *GALT* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P156 GALT.
- 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.

***GALT* mutation database:** https://databases.lovd.nl/shared/genes/GALT. We strongly encourage users to deposit positive results in the LOVD Database. 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 *GALT* exons 3 and 5 but not exon 4) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P156-B3 GALT

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*GALT*
64-105	Control fragments – see table in probemix content section for more information		
140	Reference probe 01061-L00727	12q15	
148	Reference probe 05795-L05242	15q15	
154	**GALT probe** 05036-L04422		**Exon 7**
160	Reference probe 11004-L11675	4q22	
166	**GALT probe** 05031-L04417		**Exon 2**
172	**GALT probe** 05037-L04423		**Exon 8**
178	**GALT probe** 05345-L05032		**Exon 10**
186	Reference probe 03185-L03594	1q32	
193	**GALT probe** 05035-L04421		**Exon 6**
202	Reference probe 18560-L24870	8q24	
211	**GALT probe** 05033-L04419		**Exon 4**
220	**GALT probe** 05038-L04424		**Exon 9**
229	Reference probe 01828-L01393	16p13	
239	**GALT probe** 05034-L04420		**Exon 5**
256	**GALT probe** 10650-L11968		**Exon 3**
265 *	Reference probe 10283-L10795	2q14	
274	**GALT probe** 05040-L04426		**Exon 11**
291	**GALT probe** 05030-L04416		**Exon 1**
300 *	Reference probe 06366-L21564	18p11	
316 ¬	**IL11RA probe** 05041-L04427		**Downstream**
328 ¬	**DNAI1 probe** 08075-L07856		**Upstream**
337	Reference probe 02664-L02131	11q22	

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

* New in version B3.

¬ 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. GALT probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*GALT* exon[a]	Ligation site NM_000155.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
328 ¬	08075-L07856	*DNAI1*		ACTCCAGCCAAT-TCCTCGACACCT	132.3 kb
		start codon	*31-33 (exon 1)*		
291	05030-L04416	Exon 1	4-5	GGCCCTGCAGAT-TTTCCAGCGGAT	0.5 kb
166	05031-L04417	Exon 2	165-166	GTGCTGGTGTCA-GCTCACCGCATG	0.4 kb
256	10650-L11968	Exon 3	9 nt before exon 3	CTAGCCTATCCT-TGTCGGTAGGTG	0.2 kb
211	05033-L04419	Exon 4	379-380	ATCATCCCCTTT-TCCAAGCAAAGT	0.2 kb
239	05034-L04420	Exon 5	490-491	TTGTTGATGCAT-GGGCCTCAGTCA	0.2 kb
193	05035-L04421	Exon 6	541-542	TCTGATAGATCT-TTGAAAACAAAG	0.2 kb
154	05036-L04422	Exon 7	614-615	CAGCAGTTTCCT-GCCAGATATTGC	0.4 kb
172	05037-L04423	Exon 8	728-729	GGAACGTCTGGT-CCTAACCAGTGA	0.2 kb
220 +	05038-L04424	Exon 9	872-873	CATCATGAAGAA-GCTCTTGACCAA	0.5 kb
178	05345-L05032	Exon 10	1034-1035	TGTCCGGAAATT-CATGGTTGGCTA	0.9 kb
274	05040-L04426	Exon 11	1122-1123	CTTCCTGAGGTT-CATTACCACCTG	3.6 kb
		stop codon	*1168-1170 (exon 11)*		
316 ¬	05041-L04427	*IL11RA*		CCTAGAGGCTAT-GGACACTCTGCT	

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.

+ The K285N mutation is located within the hybridising sequence of exon 9 (220 nt probe). It is not expected that the presence of the mutation will influence this probe signal.

References

- Elsas LJ, 2nd et al. (1998). The molecular biology of galactosemia. Genet Med. 1:40-48.
- 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 P156 GALT

- Papachristoforou R et al. (2014). A Novel Large Deletion Encompassing the Whole of the Galactose-1-Phosphate Uridyltransferase (GALT) Gene and Extending into the Adjacent Interleukin 11 Receptor Alpha (IL11RA) Gene Causes Classic Galactosemia Associated with Additional Phenotypic Abnormalities. *JIMD Rep.* 12:91-8.

P156 Product history	
Version	*Modification*
B3	One reference probe has been removed and two reference probes have been replaced.
B2	Two reference probes have been replaced and two removed. In addition, the control fragments have been adjusted (QDX2).
B1	One GALT probe has been replaced, one flanking probe (DNAI1) added, 7 reference probes added or replaced, and four control fragments at 88-96-100-105 nt included.
A1	First release.

Implemented changes in the product description

Version B3-01 — 23 August 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).
- Warning added to Table 1 for new probe in version B3.
- Start and stop codon, as well as ligation sites of the probes targeting the *GALT* gene updated according to new version of the NM_ reference sequence.

Version 11 – 14 March 2018 (55)
- Information added on positive control DNA samples on page 2.
- Various minor textual changes on pages 1 and 2.

Version 10 - 31 July 2017 (55)
- Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included).
- Reference included on page 1.
- Minor textual changes.

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