Product Description SALSA® MLPA® Probemix P446-A2 GALC

To be used with the MLPA General Protocol.

Version A2. As compared to version A1, six reference probes have been replaced. For complete product history see page 5.

Catalogue numbers:
- **P446-025R:** SALSA MLPA Probemix P446 GALC, 25 reactions.
- **P446-050R:** SALSA MLPA Probemix P446 GALC, 50 reactions.
- **P446-100R:** SALSA MLPA Probemix P446 GALC, 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 P446 GALC is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GALC* gene, which is associated with Krabbe disease.

Krabbe disease is an autosomal recessive lysosomal disorder affecting the white matter of the central and peripheral nervous systems. Krabbe disease is caused by homozygous or compound heterozygous mutations in the *GALC* gene on chromosome 14q31.3. The protein encoded by this gene is galactosylceramidase, which is a lysosomal enzyme involved in the catabolism of galactosylceramide, a major lipid in myelin, kidney, and epithelial cells of the small intestine and colon.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1238/.

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 *GALC* exon numbering used in this P446-A2 GALC product description is the exon numbering from the RefSeq transcript NM_000153.4, which is identical to the NG_011853.3 sequence. The exon numbering and NM_ sequence used have been retrieved on 05/2020. 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 P446-A2 GALC contains 28 MLPA probes with amplification products between 141 and 359 nucleotides (nt). This includes 19 probes for the *GALC* gene, one probe for each exon, two probes for exon 17, and one probe for alternative exon 1 of transcript variant 4 (NM_001201402), which is located 0.2 kb upstream of exon 1 of transcript variant 1 (NM_000153). 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 Krabbe disease. 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 NA04372 and NA04517 from the Coriell Institute have been tested with this P446-A2 probemix at MRC-Holland and can be used as positive control samples to detect heterozygous *GALC* exon 11-17 deletion (30 kb) and homozygous *GALC* exon 11-17 deletion (30 kb), 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.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *GALC* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P446 GALC.
- 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.

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

Table 1. SALSA MLPA Probemix P446-A2 GALC

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	GALC
64-105	Control fragments – see table in probemix content section for more information		
141 *	Reference probe 14278-L15948	15q13	
148	**GALC probe** 19446-L25860		**Exon 1**
154	**GALC probe** 19447-L25861		**Exon 5**
160 *	Reference probe 17436-L21192	16p13	
166	**GALC probe** 19448-L25862		**Exon 10**
172 Ж	**GALC probe** 19449-SP0814-L25863		**Exon 17**
179	Reference probe 17403-L21112	3p21	
184	**GALC probe** 19450-L25864		**Exon 7**
191	**GALC probe** 19451-L26363		**Exon 4**
202	**GALC probe** 19452-L25866		**Exon 13**
208	**GALC probe** 19453-L25867		**Exon 3**
216	Reference probe 10695-L20769	6p12	
226	**GALC probe** 19455-L25869		**Exon 12**
241	**GALC probe** 19456-L27345		**Exon 2**
247	**GALC probe** 19457-L25871		**Exon 15**
256 *	Reference probe 19625-L26284	10p11	
265 Ж	**GALC probe** 19454-SP0841-L26162		**Exon 8**
270	**GALC probe** 19458-L26380		**Exon 11**
276	**GALC probe** 19459-L26381		**Exon 17**
283 *	Reference probe 09361-L19757	17p11	
292 Ж	**GALC probe** 19460-SP0815-L25874		**Exon 14**
299	**GALC probe** 19461-L25875		**Exon 6**
310 *	Reference probe 11316-L12042	12p13	
319	**GALC probe** 19462-L25876		**'Exon 1'**
328	**GALC probe** 19463-L25877		**Exon 16**
338	Reference probe 12785-L15496	2q13	
346	**GALC probe** 19464-L25878		**Exon 9**
359 *	Reference probe 18252-L24241	11p15	

* New in version A2.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.

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

Table 2. GALC probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	GALC exon[a]	Ligation site NM_000153.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*19-21 (Exon 1)*		
319 ^	19462-L25876	'Exon 1'	NM_001201402.1; 251-252	GCGTTAAAGTGC-TCCACCAGGTGA	0.2 kb
148	19446-L25860	Exon 1	17-18	TGTGACCCACAC-AATGGCTGAGTG	4.7 kb
241	19456-L27345	Exon 2	220-221	TTTAGGCAACCT-CCCGACTTCTAG	0.4 kb
208	19453-L25867	Exon 3	327-328	GTGGAAATAGGT-GGTGATGGGCAG	1.7 kb
191	19451-L26363	Exon 4	444-445	AAGAGGAATCCC-AATATTACACTC	2.0 kb
154	19447-L25861	Exon 5	474-475	TTGCCATGGTCA-TTCCCTGGATGG	2.3 kb
299	19461-L25875	Exon 6	614-613, reverse	CATTATATGACC-TCTCATTCCAAA	5.8 kb
184	19450-L25864	Exon 7	715-716	TCTGGGAGTCCA-TCTCTGCATCCA	8.0 kb
265	19454-SP0841-L26162	Exon 8	1 nt before exon 8; 799-800	TTTTTTTTTCTA-30nt spanning oligo-AAGATGCAAAGT	2.9 kb
346	19464-L25878	Exon 9	1041-1042	GAATCTCCTGTC-TGGGTATCAGGT	2.0 kb
166	19448-L25862	Exon 10	1070-1071	CACTCAGTTTAC-TCAACCTGGCTG	12.9 kb
270	19458-L26380	Exon 11	107 nt after exon 11	AACTTTTAACAG-TTTGCATATGAA	0.6 kb
226	19455-L25869	Exon 12	7 nt before exon 12	TTTTGTCTTTTC-TTTACAGAGTGA	2.1 kb
202	19452-L25866	Exon 13	1397-1398	GAGCCTGCATGA-AGATGAGCTGTT	2.1 kb
292	19460-SP0815-L25874	Exon 14	41 nt before exon 14; 1511-1512	AGAGCTTCTGAA-45nt spanning oligo-CCCATTTTTTAG	4.3 kb
247	19457-L25871	Exon 15	1767-1768	GGAAGAGTAAAT-AAAGGTGGTATT	1.5 kb
328	19463-L25877	Exon 16	1891-1892	GTGTTGAAGTTA-CAGCAAAAAAAT	5.1 kb
276	19459-L26381	Exon 17	1976-1977	TCTGTGGACAGA-CATCCCTGTGAA	0.3 kb
172	19449-SP0814-L25863	Exon 17	2290-2291; 2323-2324	ATCTTTAACATT-33nt spanning oligo-TTGGCCATACCC	
		stop codon	*2074-2076 (Exon 17)*		

^ The NM_001201402.1 sequence represents transcript variant 4, this 'Exon 1' is not present in NM_000153.4, transcript variant 1.

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.

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 P446 GALC

- Irahara-Miyana K et al. (2018). Exonic deletions in GALC are frequent in Japanese globoid-cell leukodystrophy patients. *Hum Genome Var*, 5(1), 1-4.
- Ota S et al. (2020). An early-onset neuronopathic form of acid sphingomyelinase deficiency: A SMPD1 p. C133Y mutation in the saposin domain of acid sphingomyelinase. *Tohoku J Exp Med*, 250(1), 5-11.
- Zhao S et al. (2018). Large-scale study of clinical and biochemical characteristics of Chinese patients diagnosed with Krabbe disease. Clinical genetics, 93(2), 248-254.

P446 Product history	
Version	*Modification*
A2	Six reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version A2-01 — 25 May 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 the *GALC* 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 03 – 05 January 2018 (55)* - Information added on positive control DNA samples on page 2. - Small change of probe length in Table 1 in order to better reflect the true length of the amplification product. - Various minor textual changes. *Version 02 – 06 December 2016 (55)* - Product description adapted to a new lot (lot number added, small changes in Table 1 and Table 2, new picture included). *Version 01 (53)* - Not applicable, new document.

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