Product Description
SALSA® MLPA® Probemix P338-B2 GBA

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, one reference probe has been replaced and one reference probe has been removed. For complete product history see page 7.

Catalogue numbers:
- **P338-025R:** SALSA MLPA Probemix P338 GBA, 25 reactions.
- **P338-050R:** SALSA MLPA Probemix P338 GBA, 50 reactions.
- **P338-100R:** SALSA MLPA Probemix P338 GBA, 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.

This SALSA MLPA probemix is for basic research and intended for experienced MLPA users only! This probemix enables you to quantify genes or chromosomal regions in which the occurrence of copy number changes is not yet well-established and the relationship between genotype and phenotype is not yet clear. Since it will not provide you with clear cut answers, interpretation of results can be complicated. MRC Holland recommends thoroughly screening any available literature. Suggestions from specialists for improvement of this product or product description are highly appreciated.

General information
The SALSA MLPA Probemix P338 GBA is a **research use only (RUO)** assay for the detection of deletions or duplications in the *GBA* gene, which is associated with Gaucher disease. This probemix can also be used to detect the presence of the 55-bp deletion (c.1265_1319del) in *GBA* exon 9 and the c.1448T>C point mutation (p.L444P; also known as p.L483P) in *GBA* exon 10.

Gaucher disease is an autosomal recessive lysosomal storage disorder that is caused by a deficiency in beta-glucocerebrosidase enzyme activity. Beta-glucocerebrosidase is a lysosomal protein that cleaves the beta-glucosidic linkage of glucosylceramide (GlcCer, glucosylcerebroside), an intermediate in glycolipid metabolism. As a result of beta-glucocerebrosidase deficiency, there is intracellular accumulation of glucosylceramide primarily within cells of mononuclear phagocyte origin. This accumulation of fatty materials transforms the cells into so-called 'Gaucher cells' with a characteristic crumpled tissue paper appearance. Beta-glucocerebrosidase is encoded by the *GBA* gene on chromosome 1q22. Defects in the *GBA* gene are the main cause of Gaucher disease.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1269/.

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 *GBA* exon numbering used in this P338-B2 GBA product description is the exon numbering from the NM_000157.4 sequence. This *GBA* exon numbering is commonly used in literature, but differs from the *GBA* exon numbering in the NG_009783 sequence. 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 P338-B2 GBA contains 23 MLPA probes with amplification products between 155 and 363 nucleotides (nt). This includes nine probes for the *GBA* gene and three flanking probes. The *GBA* exon 9 probe will generate a lower signal when the 55-bp deletion (c.1265_1319del) is present. The *GBA* exon 10 probe will generate a lower signal when the c.1448T>C mutation (p.L444P; also known as L483P) is present. In addition, 11 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 Gaucher disease . 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. Sample ID number NA20273 from the Coriell Institute has been tested with the P338-B probemix at MRC Holland and can be used as a positive control sample to detect an absence of *GBA* exon 10. 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.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.

P338 specific notes:
- The *GBA* gene has a pseudogene, *GBAP1*, with 96% sequence identity that is located 16 kb centromeric of *GBA* (Horowitz et al. 1989). Reciprocal and nonreciprocal recombination between *GBA* and *GBAP1* can result in gene conversion or gene fusion events, resulting in the formation of recombinant alleles (Tayebi et al. 2003).
- Reduced signals for probes targeting *GBA* can be due to deletions in *GBA*, gene conversions, mutations, or the presence of recombinant alleles (Amico et al. 2016). Follow-up studies are therefore needed to determine the cause of a reduced probe signal.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *GBA* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P338 GBA.
- 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/GBA. 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 *GBA* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P338-B2 GBA

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*GBA*	Flanking
64-105	Control fragments – see table in probemix content section for more information			
155	Reference probe 09506-L23257	12p		
166 ∞ Δ	**GBA probe** 13294-L13669		**Exon 9**	
175	Reference probe 10416-L12862	9q		
181	Reference probe 16888-L20120	18q		
189	**GBA probe** 20964-L13673		**Exon 3**	
196	Reference probe 12539-L13589	13q		
203	Reference probe 10873-L11543	15q		
213 Δ	**GBA probe** 12590-L23141		**Exon 4**	
220	**GBA probe** 12584-L13665		**Upstream**	
230 ¬	**CKS1B probe** 16216-L22931			centromeric of *GBA*
238	Reference probe 08109-L07985	11p		
247	**GBA probe** 12582-L13663		**Exon 7**	
265	Reference probe 11891-L12691	8q		
283	**GBA probe** 12589-L13670		**Exon 1**	
292	**GBA probe** 20966-L13672		**Exon 8**	
301 ¬	**PKLR probe** 06516-L06074			telomeric of *GBA*
310	Reference probe 12442-L13443	14q		
319	Reference probe 10677-L11259	6p		
325	**GBA probe** 20965-L13668		**Exon 6**	
337 ¬	**CLK2 probe** 09162-L14640			telomeric of *GBA*
346	Reference probe 09073-L22672	19p		
355 ∞	**GBA probe** 12585-L13666		**Exon 10**	
363 *	Reference probe 10070-L10494	8q		

[a] See section Exon numbering on page 2 for more information.

* New in version B2.
∞ Wild type sequence detected. The presence of the 55-bp deletion (c.1265_1319del; rs80356768) will result in a decreased signal of the 166 nt probe. The presence of the c.1448T>C mutation (p.L444P; also known as L483P; rs421016) mutation will result in a decreased signal of the 355 nt probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
¬ 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.

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. *GBA* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*GBA* exon[a]	Ligation site NM_000157.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
301 ¬	06516-L06074	*PKLR*		ACAGTCTCCCAT-TCTCATATGTAG	16.1 kb
337 ¬	09162-L14640	*CLK2*		ATGTGTGAGAAA-TACAAGTTTACT	29.5 kb
		start codon	*138-140 (Exon 1)*		
220	12584-L13665	Upstream	3.4 kb before exon 1 (NM_001005741.3; 12-13, exon 1)	ATCCTGCCTTCA-GAGTCTTACTGC	3.3 kb
283 #	12589-L13670	Exon 1	38 nt before exon 1	TGTCATGTGACG-CTCCTAGTCATC	1.3 kb
	No probe	*Exon 2*			
189	20964-L13673	Exon 3	299-300	AGCTCGGTGGTG-TGTGTCTGCAAT	0.3 kb
213 Δ #	12590-L23141	Exon 4	507-508	TTGGAGGGGCCA-TGACAGATGCTG	1.6 kb
	No probe	*Exon 5*			
325 #	20965-L13668	Exon 6	891-892	GGGCCAGATACT-TTGTGAAGTAAG	0.5 kb
247	12582-L13663	Exon 7	42 nt before exon 7, reverse	CAAGAAAGTGGA-CCAGACCAGCTG	1.2 kb
292 #	20966-L13672	Exon 8	1163-1164	GAAGCAGCTAAA-TATGTTCATGGC	0.7 kb
166 ∞ Δ	13294-L13669	Exon 9	1430-1431	GGAGGACCCAAT-TGGGTGCGTAAC	0.5 kb
355 ∞ #	12585-L13666	Exon 10	1585-1584, reverse	CCACTGCGTCCA-GGTCGTTCTTCT	254.6 kb
	No probe	*Exon 11*			
		stop codon	*1746-1748 (Exon 11)*		
230 ¬	16216-L22931	*CKS1B*		TTCTGTTACAGA-CATGTCATGCTG	

[a] See section Exon numbering on page 2 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.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
∞ Wild type sequence detected. The presence of the 55-bp deletion (c.1265_1319del; rs80356768) will result in a decreased signal of the 166 nt probe. The presence of the c.1448T>C mutation (p.L444P; also known as L483P; rs421016) mutation will result in a decreased signal of the 355 nt probe.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
¬ 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.

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

P159 GLA	Fabry disease, contains probes for the *GLA* gene.
P193 NPC1-NPC2-SMPD1	Niemann-Pick disease, contains probes for the *NPC1*, *NPC2* and *SMPD1* genes.
P446 GALC	Krabbe disease, contains probes for the *GALC* gene.

References

- Amico G et al. (2016). MLPA-based approach for initial and simultaneous detection of GBA deletions and recombinant alleles in patients affected by Gaucher Disease. *Mol Genet Metab*. 119:329-337.
- Horowitz M et al. (1989). The human glucocerebrosidase gene and pseudogene: structure and evolution. *Genomics*. 4:87-96.
- 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.
- Tayebi et al. (2003). Reciprocal and nonreciprocal recombination at the glucocerebrosidase gene region: implications for complexity in Gaucher disease. *Am J Hum Genet*. 72:519-534.
- 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 P338 GBA

- Amico G et al. (2016). MLPA-based approach for initial and simultaneous detection of GBA deletions and recombinant alleles in patients affected by Gaucher Disease. *Mol Genet Metab*. 119:329-337.
- Basgalupp SP et al. (2018). Use of a multiplex ligation-dependent probe amplification method for the detection of deletions/duplications in the GBA1 gene in Gaucher disease patients. *Blood Cells Mol Dis*. 68:17-20.
- Ortiz-Cabrera NV et al. (2016). Nine-year experience in Gaucher disease diagnosis at the Spanish reference center Fundación Jiménez Díaz. *Mol Genet Metab Rep*. 9:79-85.
- Zampieri S et al. (2021). Accurate Molecular Diagnosis of Gaucher Disease Using Clinical Exome Sequencing as a First-Tier Test. *Int J Mol Sci*, 22(11), 5538.

P338 product history	
Version	*Modification*
B2	One reference probe has been replaced and one reference probe has been removed.
B1	First commercial release.

Implemented changes in the product description

Version B2-01 – 09 May 2022 (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).

Version B1-01 — 05 July 2019 (02P)
- Product description rewritten and adapted to a new template.
- Information added about the *GBAP1* pseudogene and recombinant alleles.
- Warning added in Table 1 and 2 for the 166 nt probe 13294-L13669.
- Ligation sites of the probes targeting the *GBA* gene updated according to new version of the NM_ reference sequence.
- Related SALSA MLPA probemixes added.

Version 08 — 26 April 2018 (55)
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- A reference was added.

Version 07 — 14 November 2016 (55)
- References added.

Version 06 — 18 August 2016 (55)
- Not applicable, new document.

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