Product Description
SALSA® MLPA® Probemix P022-B2 PLP1

To be used with the MLPA General Protocol.

Version B2
For complete product history see page 7.

Catalogue numbers:
- **P022-025R:** SALSA MLPA Probemix P022 PLP1, 25 reactions.
- **P022-050R:** SALSA MLPA Probemix P022 PLP1, 50 reactions.
- **P022-100R:** SALSA MLPA Probemix P022 PLP1, 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.

General information
The SALSA MLPA Probemix P022 PLP1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PLP1* gene and the Xq22 region, which are associated with *PLP1* disorders ranging from Pelizaeus-Merzbacher disease (PMD) to spastic paraplegia type 2 (SPG2).

PLP1 disorders are rare X-linked neurological disorders caused by pathogenic variants in the *PLP1* gene and resulting in defective central nervous system myelination. The *PLP1* gene encodes a transmembrane proteolipid protein that is the predominant component of myelin in the central nervous system. It may play a role in the compaction, stabilization, and maintenance of myelin sheaths, as well as in oligodendrocyte development and axonal survival. PMD (OMIM #312080) and SPG2 (OMIM #312920) are at opposite ends of the clinical spectrum of *PLP1* disorders, PMD being more severe than SPG2. PMD symptoms can begin in infancy and include amongst others nystagmus, cognitive impairment, progressive spasticity, ataxia and hypotonia, with a shortened lifespan. SPG2 manifests as spastic paraparesis, usually with a normal life span.

The *PLP1* gene (8 exons) spans ~16 kb of genomic DNA and is located on chromosome Xq22.2, about 103 Mb from the p-telomere. Complete duplication of the *PLP1* gene can be found in up to up to 70% of the PMD cases, whereas deletions of this gene as well as point mutations in coding or splice site regions are involved in the majority of the remaining cases. Heterozygous females with a *PLP1* duplication are usually asymptomatic.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1182/.

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 *PLP1* exon numbering used in this P022-B2 PLP1 product description is the exon numbering from the NG_008863.2 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the NG sequences. 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 P022-B2 PLP1 contains 37 MLPA probes with amplification products between 142 and 445 nucleotides (nt). This includes seven probes for the *PLP1* gene, one probe for each exon of the gene with the exception of exon 1, and 20 probes for the Xq22 region. In addition, ten reference probes are included that detect locations on the X-chromosome. 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 of the same sex 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 *PLP1* disorders. It is recommended to use samples of the same sex to facilitate interpretation. 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 numbers NA11005 and NA13434 from the Coriell Institute has been tested with this P022-B2 probemix at MRC Holland and can be used as positive control samples to detect a duplication of *PLP1* and part of the surrounding Xq22 region (from *BEX4* to *TMSB15B*; see Table 2) (NA11005; male) and a deletion in *PLP1* exon 5 (NA13434; male). 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:

Copy Number status: Male samples	Final ratio
Normal	0.80 < FR < 1.20
Deletion	FR = 0
Duplication	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Female samples	Final ratio
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.

Limitations of the procedure

- The SALSA MLPA Probemix P022 PLP1 will not detect small (point) mutations in the *PLP1* gene, which are the second most common cause of genetic defects in *PLP1*.
- 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.

***PLP1* mutation database**

https://databases.lovd.nl/shared/genes/PLP1. 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 *PLP1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P022-B2 PLP1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*PLP1*	Xq22
64-105	Control fragments – see table in probemix content section for more information			
142	Reference probe 19373-L25780	Xp11		
148 «	**ESX1 probe** 15338-L17172			**Telomeric**
154	**NXF3 probe** 15339-L17173			Centromeric
168	**PLP1 probe** 15345-L17179		**Exon 2**	
175	**BEX4 probe** 15341-L18181			Centromeric
181	**RAB40AL probe** 15342-L18182			Centromeric
188	**PLP1 probe** 15364-L18183		**Exon 5**	
195 «	**RAB9B probe** 15344-L18184			**Telomeric**
202	**FAM199X probe** 15346-L17180			**Telomeric**
208	**NGFRAP1 probe** 15347-L17181			Centromeric
214	Reference probe 07648-L07354	Xp11		
220	**TMSB15B probe** 15348-L17182			**Telomeric**
227	**PLP1 probe** 15349-L18185		**Exon 4**	
241	**MORF4L2 probe** 15351-L18187			Centromeric
247	Reference probe 12599-L13683	Xq12		
256	**PLP1 probe** 15366-L17200		**Exon 6**	
265	**BEX2 probe** 16013-L17194			Centromeric
274	**BHLHB9 probe** 15353-L17187			Centromeric
283	**PLP1 probe** 15354-L17188		**Exon 7**	
292	Reference probe 06037-L05492	Xq13		
301	**ARMCX5 probe** 15355-L17189			Centromeric
310	**PLP1 probe** 15356-L17190		**Exon 3**	
319	**TCEAL1 probe** 15357-L17191			Centromeric
328	**IL1RAPL2 probe** 15358-L17192			**Telomeric**
337	Reference probe 05121-L04511	Xq26		
346	**RAB40A probe** 15359-L17193			Centromeric
355	Reference probe 13524-L14330	Xq21		
364	**PLP1 probe** 15361-L18375		**Exon 8**	
373	**GLRA4 probe** 15362-L17196			Centromeric
384	Reference probe 13750-L15237	Xp22		
391	**TMSB15A probe** 15363-L18374			Centromeric
400	Reference probe 13114-L14333	Xp11		
409	Reference probe 07480-L07137	Xq21		
420	**MCART6 probe** 15365-L18373			**Telomeric**
427	**SERPINA7 probe** 15352-L18188			**Telomeric**
439	**TMEM31 probe** 15367-L18372			Centromeric
445	Reference probe 07090-L06670	Xp22		

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

« 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.

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. Xq22 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / Exon[a]	Ligation site NM_001128834.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
391 #	15363-L18374	*TMSB15A*		CTTTTCAGGCTA-TCTTCTAGTCAA	87.2 kb
301	15355-L17189	*ARMCX5*		TGACCAGGACAC-ATACAGTGACCT	147.2 kb
274 #	15353-L17187	*BHLHB9*		ACCCTAATCCTA-AACCTGTGAGCA	187.9 kb
181 #	15342-L18182	*RAB40AL*		TGCAGGACGGCA-CGGCCGAGTCCC	147.4 kb
154	15339-L17173	*NXF3*		ATCAAGTTGTTC-AAAGAAGAGCAA	131.5 kb
175	15341-L18181	*BEX4*		GGCATATTGAGC-ACAATGAAGCGA	93.1 kb
265	16013-L17194	*BEX2*		GTAAACGTTTTG-ATGTTACCTATT	68.5 kb
208	15347-L17181	*NGFRAP1*		GCCAGCTTCTAT-TTGAAGATTGCC	121.8 kb
346 #	15359-L17193	*RAB40A*		CTCACGGGAACC-ATGCTGCTTGGG	130.2 kb
319	15357-L17191	*TCEAL1*		GACCGATGAGGA-GAGGCCTCCGGT	56.8 kb
241	15351-L18187	*MORF4L2*		ATTGGCTGAAGG-GCATACGCCCTC	25.6 kb
439	15367-L18372	*TMEM31*		CAACAGTCAGAA-CAGGCAAGTGGT	9.9 kb
373	15362-L17196	*GLRA4*		GTTTGAGTGGCT-GGAAGATGCTCC	54.8 kb
		start codon	*286-288 (Exon 2)*		
	No probe	***PLP1*** Exon 1			
168	15345-L17179	***PLP1*** Exon 2	4 nt after exon 2	AGACATGGGTAA-GTTTCAAAAACT	8.6 kb
310	15356-L17190	***PLP1*** Exon 3	293-294	TTCCCCAGGCTT-GTTAGAGTGCTG	1.0 kb
227	15349-L18185	***PLP1*** Exon 4	596-597	CTTTGGCGACTA-CAAGACCACCAT	1.2 kb
188	15364-L18183	***PLP1*** Exon 5	14 nt before exon 5	TAACCACCCCAT-GTCAATCATTTT	0.7 kb
256	15366-L17200	***PLP1*** Exon 6	979-980	GCAAAACAGCTG-AGGTGAGTGGGT	0.9 kb
283	15354-L17188	***PLP1*** Exon 7	1044-1045	ACACTGGTTTCC-CTGGTGAGTTGA	1.2 kb
364	15361-L18375	***PLP1*** Exon 8	1115-1116	AGGCACCAAGTT-CTGATCCCCCGT	35.0 kb
		stop codon	*1117-1119 (Exon 8)*		
195 «	15344-L18184	*RAB9B*		TTTGTAACCCTC-CAGATCTGGGAC	139.6 kb
220 #	15348-L17182	*TMSB15B*		GGATCTCCTCCA-AAGAGCAGATTT	129.4 kb
420 #	15365-L18373	*MCART6*		TCATGTCTGGCG-TGGTGGAGGCCG	81.2 kb
202	15346-L17180	*FAM199X*		TGATGTACTTTC-TGATGTCATACC	68.7 kb
148 «	15338-L17172	*ESX1*		AGCGCTTGAAGC-ATGGAGTCTCTT	940.7 kb
328	15358-L17192	*IL1RAPL2*		TCAAGACATACA-TGGCTTTGGCAG	839.0 kb
427	15352-L18188	*SERPINA7*		CAGTGGAAGCTG-CCATGTCATCTA	

[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.

« 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.

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.

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

- P071 LMNB1-PLP1-NOTCH3 Contains probes for the *LMNB1*, *PLP1* and *NOTCH3* genes.

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 P022 PLP1

- Ji H et al. (2018). Hypomyelinating disorders in China: the clinical and genetic heterogeneity in 119 patients. *PLoS One*. 13:e0188869.
- Kim SJ et al. (2008). Identification of proteolipid protein 1 gene duplication by multiplex ligation-dependent probe amplification. *J Korean Med Sci*. 23:328-331.
- Mądry J et al. (2010). PLP1 gene duplication as a cause of the classic form of Pelizaeus-Merzbacher disease–case report. *Neurol Neurochir Pol*. 44:511-515.
- Martinez-Montero P et al. (2013). PLP1 gene analysis in 88 patients with leukodystrophy. *Clin Genet*. 84:566-571.
- Masliah-Planchon J et al. (2015). Insertion of an extra copy of Xq22.2 into 1p36 results in functional duplication of the PLP1 gene in a girl with classical Pelizaeus-Merzbacher disease. *BMC Med Genet*. 16:77.
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- Shimojima K et al. (2012). Pelizaeus-Merzbacher disease caused by a duplication-inverted triplication-duplication in chromosomal segments including the PLP1 region. *Eur J Med Genet*. 55:400-403.
- Vieira AMP et al. (2021). Subclinical hypothyroidism and Pelizaeus-Merzbacher Disease in same-sex twins: Case report. *J Clin Transl Endocrinol Case Rep*. 22:100097.
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- Wolf NI et al. (2005). Three or more copies of the proteolipid protein gene PLP1 cause severe Pelizaeus-Merzbacher disease. *Brain*. 128:743-751.
- Xue H et al. (2021). Prenatal diagnosis of PLP1 duplication by single nucleotide polymorphism array in a family with Pelizaeus-Merzbacher disease. *Aging (Albany NY)*. 13:1488-1497.

P022 product history	
Version	*Modification*
B2	One reference probe has been removed and one reference probe has been replaced. In addition, the control fragments have been adjusted (QDX2).
B1	Probemix completely redesigned.
A1	First release.

Implemented changes in the product description
Version B2-03 – 01 February 2022 (04P) - Product description rewritten and adapted to a new template. - *General information* section updated. - Two positive samples added to the *Positive control DNA samples* section. - NM_ reference sequence of the *PLP1* gene updated. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. - Warning added to Table 1 and 2 for probes located in or near a GC-rich region. - One additional warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - List with selected publications using SALSA MLPA Probemix P022 PLP1 updated. Version B2-02 — 16 October 2019 (02P) - Product description adapted to a new template. - GeneReviews link for more information corrected in the *General Information* section. - Link to the *PLP1* mutation database corrected. - Warning about off-scale peaks added to the *Interpretation of results* section. - *Related SALSA MLPA probemixes* section updated. - List with selected publications using SALSA MLPA Probemix P022 PLP1 updated. - Various minor textual or layout changes. Version B2-01 – 24 May 2018 (01P) - Product description restructured and adapted to a new template. Version 15 – 16 March 2018 (55) - Information added on positive control DNA samples on page 2. - New reference added on page 2. - Ligation sites of the probes targeting the *PLP1* gene updated according to new version of the NM_ reference sequence. - Various minor textual and layout changes. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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