Instructions for Use

SALSA® MLPA® Probemix P405 CMT1

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 P405 CMT1 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	SALSA® MLPA® Probemix P405 CMT1
Version	B1
Catalogue numbers	P405-025R (25 reactions) P405-050R (50 reactions) P405-100R (100 reactions)
Basic UDI-D	872021148P40562
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

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

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.

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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

Changes in this Product Version:
As compared to version A1, four reference probes and three target probes have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P405 CMT1 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 human *PMP22* gene and deletions in the *GJB1* gene in genomic DNA isolated from human peripheral whole blood specimens. P405 CMT1 is intended to confirm a potential cause for Charcot-Marie-Tooth disease type 1A (CMT1A) (duplications in *PMP22*) and X-linked CMT (CMT1X) (deletions in *GJB1*) or confirm a potential cause for and clinical diagnosis of hereditary neuropathy with liability to pressure palsies (HNPP) (deletions in *PMP22*) and for molecular genetic testing of at-risk family members.[3]

Copy number variations (CNVs) detected with P405 CMT1 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 *GJB1*, and some defects in *PMP22* 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.

[3]Certain probes targeting additional genes included in P405 may only be used in a research setting. The following table summarises which probes are for IVD or exclusively restricted to RUO use:

IVD targets	RUO targets
PMP22, GJB1	*TEKT3, COX10, MPZ*

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 CMT or HNPP. • Should be from the same sex to facilitate interpretation.	
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, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• In the validation experiments of this probemix, DNA samples from healthy individuals of the same sex should be used.	

*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	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 Probes Targeting Two Copies (*MPZ*, *PMP22* and 17p12 region)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

Typical Results of *GJB1* X Probes (Compared to Same Gender)

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 P405 CMT1 in 67 samples from healthy individuals with normal copy numbers and nine samples with known CNVs in *PMP22* or *GJB1*. 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 P405 CMT1 on three samples with known CNVs and on one sample without any mutation 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 P405 CMT1 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. For most probes, expected FRs were obtained even in the presence of potential interferents at concentrations shown in the table below.

<table>
<tr><td></td><td>
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 154/156 probes</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 155/156 probes</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 155/156 probes</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 155/156 probes</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 73/156 probes</td></tr>
</table>
* Results are summarised for all probes across all four samples tested.

An effect on the FRs was observed for a low number of probes with EDTA, NaCl, Fe^{3+}, and heparin. The effect was mild as only ambiguous ratios were obtained, potentially leading only to delayed results. Hemoglobin had the largest effect on the FRs, leading to false deletions.

Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect.

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.
</td></tr>
<tr><td>Cross-reactivity</td><td>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 on nine samples with known copy number variations and 45 normal samples to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, three previously genotyped samples were tested using SALSA MLPA Probemix P405 CMT1 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 585/585 data points, leading to a precision of 100%.</td></tr>
<tr><td>Clinical validity*</td><td>PMP22: 20-64% of CMT1 is caused by duplications in PMP22. 80% of HNPP is caused by deletions in PMP22 (van Paassen et al. 2014).

GJB1: 0.3% of CMT1X is caused by deletions in GJB1 (Ainsworth et al. 1998, Capponi et al. 2015, Gonzaga-Jauregui et al. 2010, Nakagawa et al. 2001, Takashima et al. 2003).

*(Based on a 2000-2024 literature review)</td></tr>
</table>

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
Xp22	X Chromosome		27.3 **M**b	384	13750-L15237	§
Xp11.4	X Chromosome		32.3 **M**b	328	13522-L14327	§
Xq13.1	*GJB1*	Upstream (Exon 1)	7.6 kb	196	13202-L14523	≠
Xq13.1	*GJB1*	Exon 1 (2)	0.5 kb	391	22646-L31861	
Xq13.1	*GJB1*	Exon 2	0.4 kb	136	02629-L02096	
Xq13.1	*GJB1*	Exon 2	0.7 kb	436	06188-L20735	
Xq13.1	*GJB1*	Exon 2	58.7 **M**b	213	13203-L19630	
Xq25	X Chromosome			418	00820-L20737	§
1q23.3	*MPZ*	Exon 6	0.2 kb	301	04900-L04284	
1q23.3	*MPZ*	Exon 5	0.3 kb	270	04899-L20744	
1q23.3	*MPZ*	Exon 4	0.4 kb	232	04898-L17028	
1q23.3	*MPZ*	Exon 3	0.5 kb	208	04897-L32050	
1q23.3	*MPZ*	Exon 2	2.6 kb	178	04896-L04280	
1q23.3	*MPZ*	Exon 1	0.2 kb	165	04895-L31858	
1q23.3	*MPZ*	Exon 1		226	06139-L05583	
17p12	*ELAC2*		1.2 **M**b	346	01466-L00917	¬
17p12	*COX10*	CMT1 region	0.2 kb	427	01469-L20736	Ø
17p12	*COX10*	CMT1 region	1.0 **M**b	400	01468-L19633	Ø
17p12	*PMP22*	Exon 5	8.6 kb	244	04659-L19632	
17p12	*PMP22*	Exon 4	0.1 kb	311	22650-L31865	
17p12	*PMP22*	Exon 4	19.5 kb	172	11539-L04463	
17p12	*PMP22*	Exon 3	0.1 kb	203	22645-L31860	
17p12	*PMP22*	Exon 3	1.5 kb	148	04657-L04461	
17p12	*PMP22*	Exon 2	4.5 kb	256	01462-L00927	
17p12	*PMP22*	Exon 1	0.1 kb	238	22648-L31863	
17p12	*PMP22*	Exon 1	2.3 kb	142	04656-L04039	
17p12	*PMP22*	Upstream	4.2 kb	355	22651-L31866	Ø
17p12	*PMP22*	Upstream	5.9 kb	373	02729-L02156	Ø
17p12	*PMP22*	Upstream	26.4 kb	184	02678-L02158	Ø
17p12	*TEKT3*	CMT1 region	27.4 kb	154	22647-L32049	∫
17p12	*TEKT3*	CMT1 region	2.7 **M**b	293	22649-L32051	∫
17p11.2	*DRC3*			278	01452-L20745	¬
2p	Reference			364	05953-L05397	
3p	Reference			319	15385-L17792	
4q	Reference			445	12526-L13576	
5q	Reference			130	00797-L13645	
6q	Reference			220	14968-L16704	
7p	Reference			190	06148-L04992	
12q	Reference			160	07394-L07041	
14q	Reference			409	02718-L00732	
16q	Reference			250	18056-L31125	
20q	Reference			286	07737-L21372	

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 *MPZ, PMP22* and *GJB1* exon numbers are from the MANE Select project and are based on MANE Select transcripts. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of one probe with a target at the edge of or slightly outside the coding region is altered. The exon numbering from the previous version of this Product Description is disclosed between brackets when a discrepancy is present.
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.

Ø These probes target sequences outside of the known coding region. Copy number alterations of only these probes are of unknown clinical significance.

§ X-chromosome probe. Used for the determination of X-chromosome copy number.

≠ This probe targets a non-coding sequence near the *GJB1* gene. Copy number alterations of only this probe are of unknown clinical significance.

∫ The clinical significance of the following findings is not yet clear/clearly established: recurrent duplications have been described which can be detected by the two TEKT3 probes, but not by any of the PMP22 probes. These duplications may cause CMT through an unknown mechanism affecting *PMP22* expression (Weterman et al. 2010, Zhang et al. 2010).

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.
5. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.
6. Mosaicism concerning *PMP22* has been reported in individuals with CMT1A. Mosaic *PMP22* results obtained with the P405 probemix must be confirmed by analysis of a second, independently collected DNA sample or a different technique, in order to exclude a false positive mosaic result. (Ferese et al. 2021, Slater et al. 2004).

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. Target probes for *MPZ*, *COX10*, and *TEKT3* CNVs are included to be used for research purposes only and not for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Ainsworth PJ et al. (1998). Genotype/phenotype correlation in affected individuals of a family with a deletion of the entire coding sequence of the connexin 32 gene. Hum Genet. 103:242-244.
2. Capponi S et al. (2015). Contribution of copy number variations in CMT 1X: a retrospective study. European Journal of Neurology. 22:406-409.
3. Ferese R et al. (2021). Cohort Analysis of 67 Charcot-Marie-Tooth Italian Patients: Identification of New Mutations and Broadening of Phenotype Expression Produced by Rare Variants. Front Genet. 12:682050.
4. Gonzaga-Jauregui C et al. (2010). GJB1/Connexin 32 whole gene deletions in patients with X-linked Charcot-Marie-Tooth disease. Neurogenetics. 11:465-470.
5. Nakagawa M et al. (2001). Clinical phenotype in X-linked Charcot-Marie-Tooth disease with an entire deletion of the connexin 32 coding sequence. J Neurol Sci. 185:31-37.
6. Slater H et al. (2004). Improved testing for CMT1A and HNPP using multiplex ligation-dependent probe amplification (MLPA) with rapid DNA preparations: comparison with the interphase FISH method. Human mutation. 24:164-171.
7. Takashima H et al. (2003). Gap junction protein beta 1 (GJB1) mutations and central nervous system symptoms in X-linked Charcot-Marie-Tooth disease. Acta Neurol Scand. 107:31-37.
8. van Paassen BW et al. (2014). PMP22 related neuropathies: Charcot-Marie-Tooth disease type 1A and Hereditary Neuropathy with liability to Pressure Palsies. Orphanet J Rare Dis. 9:38.
9. Weterman MA et al. (2010). Copy number variation upstream of PMP22 in Charcot–Marie–Tooth disease. European journal of human genetics. 18:421-428.
10. Zhang F et al. (2010). Mechanisms for nonrecurrent genomic rearrangements associated with CMT1A or HNPP: rare CNVs as a cause for missing heritability. Am J Hum Genet. 86:892-903.

Implemented Changes in the Product Description

Version B1-04 – 25 January 2025 (03S)
- The product description has been updated to a new template.
- Intended purpose has been updated, *COX10*, *TEKT3*, *MPZ* genes removed.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Exon numbering updated for GJB1 probes 13202-L14523 and 22646-L31861.
- Warning for probe target outside the known coding region added for probe 13202-L14523.
- Precaution relating to mosaicism added.
- Probemix is now IVDR certified.