## Instructions for Use

# SALSA® MLPA® Probemix P033 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 P033 CMT1 product page on our website to find Certificates of Analysis and a list of related products.

| **Product Name** | **SALSA® MLPA® Probemix P033 CMT1** |
|---|---|
| **Version** | **B4** |
| **Catalogue numbers** | **P033-025R (25 reactions)**<br>**P033-050R (50 reactions)**<br>**P033-100R (100 reactions)** |
| **Basic UDI-DI** | **872021148P0335K** |
| **Ingredients** | **Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA** |

| **Additional Test Components** | **Catalogue numbers** |
|---|---|
| SALSA® MLPA® Reagent Kit | EK1-FAM<br>EK1-CY5<br>EK5-FAM<br>EK5-CY5<br>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<br>COLOMBIA<br>ISRAEL<br>COSTA RICA |
| 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<br>1057 DL, Amsterdam, the Netherlands |
| E-mail | info@mrcholland.com (information & technical questions);<br>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 B3, three reference probes have been replaced.

## 1. Intended Purpose

The SALSA MLPA Probemix P033 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 in genomic DNA isolated from human peripheral whole blood specimens. P033 CMT1 is intended to confirm a potential cause for Charcot-Marie-Tooth disease type 1A (CMT1A) (duplications in *PMP22*) 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 P033 CMT1 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. 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 P033 may only be used in a research setting. The following table summarises which probes are for IVD or exclusively restricted to RUO use:

| IVD target | RUO targets |
|---|---|
| *PMP22* | *KIF1b, TEKT3, COX10* |

## 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:<br>• QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)<br>• Promega Wizard Genomic DNA Purification Kit (manual)<br>• Salting out (manual) |

| Sample Types | | |
|---|---|---|
| Test Sample | • Provided by user | |
| Reference Samples (Required) | • Provided by user<br>• Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples.<br>• Have a normal copy number and ≤0.10 standard deviation for all probes.<br>• 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. | |
| No-DNA Control (Preferably) | • Provided by user<br>• $TE_{0.1}$ buffer instead of DNA<br>• 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. |

*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 (*PMP22* and 17p12 region, *KIF1b*)

| 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 |

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 can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P033 CMT1 in 46 samples from healthy individuals with normal copy numbers and nine samples with known CNVs in *PMP22*. 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 P033 CMT1 on two 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.<br><br>A study using SALSA MLPA Probemix P033 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. |

| Interferent | Source | Testing Concentration | Results* |
|---|---|---|---|
| **EDTA** | Exogenous – specimen collection tubes | 1.5 mM | Copy number: Expected FR for 52/54 probes |
| **NaCl** | Exogenous – DNA extraction | 40 mM | Copy number: Expected FR for 52/54 probes |
| **$Fe^{3+}$ ($FeCl_3$)** | Exogenous – DNA extraction | 1 µM | Copy number: Expected FR for 54/54 probes |
| **Heparin** | Exogenous – specimen collection tubes | 0.02 U/mL | Copy number: Expected FR for 54/54 probes |

| | |
|---|---|
| | **Hemoglobin** Endogenous – blood sample 0.02 µg/µl Copy number: Expected FR for 30/54 probes<br>* Results are summarised for all probes across all three samples tested.<br>Endogenous (hemoglobin) and exogenous interfering substances (EDTA, heparin, salts (NaCl), and FeCl3) were tested and shown to have mild effects on P033 CMT1 results. At most, these substances led to ambiguous ratios and potential delayed results in the case of NaCl, EDTA, and hemoglobin. None of the interferents tested had an effect leading to a potential false result. Neither $FeCl_3$ nor heparin had any effect on probe ratios.<br>Additionally, Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores.<br>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. |
| Cross-reactivity | 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 23 normal samples to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity. |
| Accuracy | Results of accuracy are derived from trueness and precision studies. For trueness, four previously genotyped samples were tested using SALSA MLPA Probemix P033 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 396/405 data points, leading to a precision of 98%. |
| Clinical validity* | 20-64% of CMT1 is caused by duplications in *PMP22*. 80% of HNPP is caused by deletions in *PMP22* (van Paassen et al. 2014).<br>*(Based on a 2015-2024 literature review) |

**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 |
|---|---|---|---|---|---|---|
| 1p36.22 | *KIF1B* | Exon 2 | 142.9 kb | 154 | 04681-L04462 | |
| 1p36.22 | *KIF1B* | Exon 48 (46) | | 220 | 04682-L04060 | |
| 17p12 | *ELAC2* | | 1.2 **M**b | 346 | 01466-L00917 | ¬ |
| 17p12 | *COX10* | CMT1 region | 0.2 kb | 418 | 01469-L00924 | Ø |
| 17p12 | *COX10* | CMT1 region | 1.0 **M**b | 391 | 01468-L00925 | Ø |
| 17p12 | *PMP22* | Exon 5 | 0.1 kb | 239 | 04659-L04464 | |
| 17p12 | *PMP22* | Exon 5 | 8.5 kb | 337 | 01465-L00930 | Δ |
| 17p12 | *PMP22* | Exon 4 | 0.1 kb | 310 | 02145-L01641 | |
| 17p12 | *PMP22* | Exon 4 | 19.5 kb | 172 | 11539-L04463 | |
| 17p12 | *PMP22* | Exon 3 | 0.1 kb | 166 | 04658-L04041 | |
| 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 | 229 | 01461-L00926 | |
| 17p12 | *PMP22* | Exon 1 | 2.3 kb | 142 | 04656-L04039 | |
| 17p12 | *PMP22* | Upstream | 4.2 kb | 355 | 02730-L02157 | 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 | 27.3 kb | 202 | 01460-L00921 | § ∫ |
| 17p12 | *TEKT3* | CMT1 region | 2.7 **M**b | 292 | 04660-L02155 | ∫ |
| 17p11.2 | *DRC3* | | | 274 | 01452-L00936 | ¬ |
| 1p | Reference | | | 302 | 06487-L06013 | |
| 1q | Reference | | | 400 | 13588-L15045 | |
| 2p | Reference | | | 160 | 00822-L00130 | |
| 5p | Reference | | | 283 | 08044-L07825 | |
| 5q | Reference | | | 130 | 00797-L00463 | |
| 6q | Reference | | | 267 | 17834-L22900 | |
| 6q | Reference | | | 328 | 14943-L16676 | |
| 7q | Reference | | | 178 | 02958-L02390 | |
| 8q | Reference | | | 427 | 17426-L21388 | |
| 8q | Reference | | | 319 | 01042-L10915 | |
| 9q | Reference | | | 381 | 19749-L26532 | |
| 11p | Reference | | | 436 | 03537-L02903 | |
| 11p | Reference | | | 193 | 00976-L00563 | |
| 12q | Reference | | | 211 | 00472-L00088 | |
| 14q | Reference | | | 364 | 15131-L16901 | |
| 17q | Reference | | | 409 | 00446-L00390 | |
| 21q | Reference | | | 247 | 00816-L00334 | |
| 21q | Reference | | | 137 | 03797-L04594 | |

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 *PMP22* and *KIF1b* 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. 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. |
| Δ | This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution. |
| Ø | These probes target sequences outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance. |
| § | This probe signal may be influenced by the presence of a 9 nt deletion including the ligation site (rs759000506). |
| ∫ | 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 P033 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 *COX10*, *TEKT3* and *KIF1B* 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. 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.
2. 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.
3. 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.
4. Weterman MA et al. (2010). Copy number variation upstream of PMP22 in Charcot–Marie–Tooth disease. European journal of human genetics. 18:421-428.
5. 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 B4-07 – 22 January 2025 (03S)*
- The product description has been updated to a new template.
- Intended purpose has been updated, *COX10*, *TEKT3*, *KIF1B* genes removed.
- Salt warning has been removed from reference probe 00822-L00130.
- Exon numbering updated for KIF1B probe 04682-L04060.
- SNV rs1907114176 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Precaution relating to mosaicism added.
- Probemix is now IVDR certified.