Instructions for Use
SALSA® MLPA® Probemix P017 MEN1

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P017 MEN1 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P017 MEN1**
Version	D1
Catalogue numbers	P017-025R (25 reactions) P017-050R (50 reactions) P017-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE ISRAEL
RUO	ALL OTHER COUNTRIES

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

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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.

More Information: www.mrcholland.com	
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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 or country in which the user and/or the patient is located.

Changes in this Product Version
As compared to version C1, one target probe has been removed and three reference probes have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P017 MEN1 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *MEN1* gene in genomic DNA isolated from human peripheral whole blood specimens. P017 MEN1 is intended to confirm a potential cause for and clinical diagnosis of multiple endocrine neoplasia type 1 (MEN1) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P017 MEN1 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 the *MEN1* gene 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, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, 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 MEN1.
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

*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 (*MEN1*)

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

The expected percentage of deletions/duplications is 1-4% of all *MEN1* mutations according to the UMD, LOVD, and CLinVar (Concolino et al. 2016, Lemos and Thakker 2008, Romanet et al. 2019).

Analytical performance for the detection of deletions/duplications in *MEN1* is very high and can be considered >99% (based on a literature review covering 2007 to 2024). Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
11q13.1	*SF1*		27.9 kb	362	17117-L20815	« ¬
11q13.1	*MEN1*	Exon 10	0.6 kb	256	01164-L20713	« »
11q13.1	*MEN1*	Exon 9	0.1 kb	154	17113-L20265	«
11q13.1	*MEN1*	Exon 9	0.6 kb	161	03404-L20714	« »
11q13.1	*MEN1*	Exon 8	0.6 kb	337	03403-L09561	«
11q13.1	*MEN1*	Exon 7	0.7 kb	301	01666-L01245	« »
11q13.1	*MEN1*	Exon 6	0.2 kb	234	17112-L20814	« »
11q13.1	*MEN1*	Exon 5	0.4 kb	202	13159-L14681	« »
11q13.1	*MEN1*	Exon 4	0.4 kb	167	13158-L14680	« »
11q13.1	*MEN1*	Exon 3	2.0 kb	283	01665-L14816	« »
11q13.1	*MEN1*	Exon 2 (Exon 2b)	0.6 kb	228	01664-L20716	« »
11q13.1	*MEN1*	Exon 1	0.2 kb	241	13157-L20717	«
11q13.1	*MEN1*	Upstream (Exon 1)	0.3 kb	195	01663-L20715	« Ø »
11q13.1	*MEN1*	Upstream	216.6 kb	355	13161-L14683	« ∫
11q13.1	*SNX15*			328	01667-L14817	¬
1q	Reference			185	03200-L03342	
4q	Reference			247	19086-L24973	
6q	Reference			274	13393-L14850	
9q	Reference			142	15097-L16868	
10q	Reference			211	16649-L19182	
12q	Reference			373	04278-L03682	
14q	Reference			310	12442-L13443	
16q	Reference			175	11571-L20271	
17q	Reference			148	09970-L10429	
18q	Reference			292	13325-L14751	

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 *MEN1* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotation 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.
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.
«	These probes are 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.
Ø	This probe targets a sequence outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance.
»	These probes detect the same sequences as MEN1 probes in SALSA MLPA Probemix P244-D1 AIP-MEN1-CDKN1B.
∫	The ligation site of this probe is located in an alternative exon 1 present in two alternative transcripts: NM_130803.2 and NM_130804.2. In both transcripts the ligation site is at position 264-265. The significance of deletions/duplications of only this alternative exon is not clear as this exon is non-coding and alternative transcript variants using other transcription start sites are known.

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. **Please note that this probemix is not suited to detect deletions or duplications in DNA extracted from fresh tumour tissue or from formalin-fixed paraffin embedded (FFPE) tumour materials. SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B (only version D1) can be used in a research setting to detect CNVs in the *MEN1*-region in DNA from tumour material.**
5. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Concolino P et al. (2016). Multiple endocrine neoplasia type 1 (MEN1): An update of 208 new germline variants reported in the last nine years. Cancer Genet. 209:36-41.
2. Lemos MC et al. (2008). Multiple endocrine neoplasia type 1 (MEN1): analysis of 1336 mutations reported in the first decade following identification of the gene. Hum Mutat. 29:22-32.
3. Romanet P et al. (2019). UMD-MEN1 Database: An Overview of the 370 MEN1 Variants Present in 1676 Patients From the French Population. J Clin Endocrinol Metab. 104:753-764.

Implemented changes in the product description

Version D1-05 – 27 March 2025 (03S)

- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Distance to next probe updated for probe 13161-L14683.
- Exon numbering updated for MEN1 probe 01664-L20716.
- A warning for a probe targeting a sequence outside of the known coding region was added for probe 01663-L20715.