Product Description
SALSA® MLPA® Probemix P244-D1 AIP-MEN1-CDKN1B

To be used with the MLPA General Protocol.

Version D1
As compared to version C1 three new target probes have been included and the length of two target probes has changed. All reference probes are replaced and the total number of reference probes has increased to twelve. For complete product history see page 11.

Catalogue numbers:
- **P244-025R:** SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B, 25 reactions.
- **P244-050R:** SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B, 50 reactions.
- **P244-100R:** SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B, 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.

Intended purpose
The SALSA MLPA Probemix P244-AIP-MEN1-CDKN1B is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in *AIP*, *MEN1* and *CDKN1B* in order to confirm a potential cause and clinical diagnosis of familial isolated pituitary adenoma (FIPA), multiple endocrine neoplasia type 1 (MEN1) or multiple endocrine neoplasia type 4 (MEN4), respectively. This assay is for use with human DNA extracted from peripheral whole blood specimens.

Copy number variations (CNVs) detected with P244 AIP-MEN1-CDKN1B 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 *AIP*, *MEN1* and *CDKN1B* genes 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, population screening, pre-implantation or prenatal testing. Only in a research setting can this device be used 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.

Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background

Multiple endocrine neoplasia type 1 (MEN1) is predominantly characterized by the occurrence of primary hyperparathyroidism (PHPT), which occurs in 95-100% of patients; pancreatic neuroendocrine tumours, which occur in 40-75% of patients; and pituitary adenoma, which is found in 30-50% of patients. Most tumours are non-metastasizing, but many can cause striking and serious clinical effects due to the increased secretion of hormones. It is estimated that in the general population 1 to 10 in 100.000 individuals develop MEN1 during their lifetime. Nine out of ten patients diagnosed with MEN1 have the familial form. MEN1 shows dominant autosomal inheritance and the penetrance is >95% by age 40 for confirmed pathogenic mutations. The mean age of death of MEN1 patients is between 50 and 55 years. The single gene associated with MEN1 syndrome is *MEN1*, which encodes the menin protein. Heterozygous *MEN1* pathogenic variants are found in ~90% of familial MEN1 syndrome patients and in ~65% of sporadic cases. Loss of heterozygosity (LOH) of MEN1 is observed in >90% MEN1 tumours suggesting that *MEN1* acts as a tumour suppressor gene, in line with the Knudson 2-hit hypothesis for tumorigenesis. Besides point mutations, several deletions involving one or more complete exons in the *MEN1* gene have been described (Carroll 2013, Concolino et al. 2016, Lemos and Thakker 2008, Romanet et al. 2019, Thakker 2014), including a pathogenic deletion of just the 5'-UTR (Kooblall et al. 2020).

Pituitary adenomas (PAs) occur with a frequency of ~1 in 1000 in the general population. Most cases are sporadic, but approximately 5% occurs as a familial cancer. The *AIP* gene encodes aryl hydrocarbon receptor-interacting protein (AIP), a tumour suppressor that is involved in the control of cell proliferation and differentiation. *AIP* loss of function mutations are found in 15-25% of familial isolated pituitary adenoma (FIPA) cases, which are subsequently referred to as *AIP*-FIPA. Inheritance is autosomal dominant and the average penetrance is 15-30%, although this may vary greatly. The prevalence of *AIP*-FIPA is estimated at 1:100,000. Similar as for *MEN1*, LOH is frequently observed, suggesting that *AIP* also acts as a tumour suppressor gene (Cai et al. 2013). Although most known germline *AIP* mutations are point mutations, several exon deletions have been reported: exon 1-2, exon 2, exon 1-6 (Georgitsi et al. 2008, Igreja et al. 2010, Marques et al. 2018).

MEN1 and *AIP* are located in close proximity on 11q13, and somatic LOH in MEN1 and FIPA associated tumours often affects both genes. Apart from tumours in MEN1 and FIPA patients, LOH of this locus also occurs in sporadic cancers, especially in endocrine tissues. As both genes are considered tumour suppressor genes this double loss may contribute to tumorigenesis. Chromosomal losses of the 11q13 chromosomal band have also been found in other cancers, such as cervical cancer and hibernomas (Newsham 1998; Nord et al. 2010).

MEN4 is a distinct MEN type but the symptoms of MEN4 largely overlap with MEN1 (Pellegata et al. 2006). In a small number (estimated at 1-3%) of *MEN1* mutation-negative patients fulfilling the diagnostic criteria for MEN1, mutations in *CDKN1B* have been detected. Extrapolating from this, the prevalence of MEN4 is very low: <1:300,000. Like MEN1, MEN4 is primarily characterized by PHPT and PA, but the additional tumours show some differences; tumours in the reproductive organs, and adrenal and renal tumours have been found in MEN4 patients. The only way to distinguish MEN4 from MEN1 is by identification of a pathogenic mutation in *CDKN1B*. Somatic mutations in *CDKN1B* have also been identified in sporadic tumours, but LOH of *CDKN1B* in MEN4-related tumours has not been found.

More information on MEN1 can be found on https://www.ncbi.nlm.nih.gov/books/NBK1538/
More information on AIP-related FIPA can be found on https://www.ncbi.nlm.nih.gov/books/NBK97965/
More information on MEN4 can be found on: https://omim.org/entry/610755

Gene structure

The *MEN1* gene spans ~7.2 kilobases (kb) on chromosome 11q13, ~2.6 Mb centromeric from *AIP*, and contains 10 exons. The *MEN1* LRG_509 is available at www.lrg-sequence.org and is identical to GenBank NG_008929.1.

The *AIP* gene spans ~8.1 kb of genomic sequence on chromosome 11q13, ~2.6 Mb telomeric from *MEN1*, and contains 6 exons. The *AIP* LRG_460 is available at www.lrg-sequence.org and is identical to GenBank NG_008969.1.

The *CDKN1B* gene spans 5.0 kb of genomic sequence on chromosome 12p13, and contains 3 exons. No LRG is available for *CDKN1B*, but the GenBank chromosomal sequence is NG_016341.1.

Transcript variants

For *MEN1,* multiple variants have been described. Transcript variant 4 encodes menin isoform 2 (NM_001370259.2, 2712nt; coding sequence 62-1894), which is the best-characterised isoform. This sequence is the MANE Select reference standard. The ATG translation start site is located in exon 2 and the stop codon is located in exon 10. More information about NM_001370259.2 and other *MEN1* transcript variants can be found on the NCBI *MEN1* gene page: www.ncbi.nlm.nih.gov/gene/4221.

For *AIP,* multiple variants have been described. Transcript variant 1 is the longest and most abundant transcript and encodes the longest AIP isoform (NM_003977.4; 1236 nt; coding sequence 134-1126). The ATG translation start site is located in exon 1 and the stop codon is located in exon 6. More information about NM_003977.4 and other *AIP* transcript variants can be found on the NCBI *AIP* gene page: https://www.ncbi.nlm.nih.gov/gene/9049.

For *CDKN1B,* one transcript variant has been described encoding the full length protein (NM_004064.5; 2411 nt; coding sequence 473-1069; http://www.ncbi.nlm.nih.gov/gene/1027). The ATG translation start site is located in exon 1 and the stop codon is located in exon 2.

Exon numbering

The *MEN1* exon numbering used in this P244-D1 AIP-MEN1-CDKN1B product description is the exon numbering from the MANE Select transcript. 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.

The *AIP* exon numbering used in this P244-D1 AIP-MEN1-CDKN1B product description is the exon numbering from the MANE Select transcript. 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.

The *CDKN1B* exon numbering used in this P244-D1 AIP-MEN1-CDKN1B product description is the exon numbering from the MANE Select transcript. 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 P244-D1 AIP-MEN1-CDKN1B contains 42 MLPA probes with amplification products between 129 and 463 nucleotides (nt). This includes 25 probes for the *MEN1-AIP* region, five probes for the *CDKN1B* region. In addition, 12 reference probes are included that target relatively copy number stable regions in all cancer types. 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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 from human peripheral blood or, in a research setting, DNA derived from fresh or FFPE tumour tissue, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 healthy individuals without a history of MEN or FIPA. 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 NA07981 from the Coriell Institute has been tested with this P244-D1 probemix at MRC Holland and can be used as a positive control sample to detect four copies of *CDKN1B* and the flanking regions. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
Deletions and duplications account for 1-4% of all pathogenic *MEN1* mutations and large deletions or duplications account for 2-5% of the pathogenic *AIP* mutations. For *CDKN1B* this percentage is not known: very few pathogenic mutations have been identified and large deletions and duplications appear to be very rare. Analytical performance for the detection of deletions/duplications in *MEN1*, *AIP* and *CDKN1B* is very high and can be considered >99% (based on a 2007-2020 literature review).

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.

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 expected results for *MEN1*, *AIP* and *CDKN1B* target probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (homozygous duplication or heterozygous triplication).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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic or subclonal 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 or in or near the *MEN1-AIP* genes. 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

- In most populations, the major cause of genetic defects in the *MEN1*, *AIP* and *CDKN1B* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumours with high chromosomal instability.

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.

Mutation databases

The UMD-MEN1 mutation database (www.umd.be/MEN1/) and the LOVD page for *MEN1*: databases.lovd.nl/shared/genes/MEN1. We strongly encourage users to deposit positive results in any of the *MEN1* databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

The FIPA Patients *AIP* Mutations Database: https://aip.fipapatients.org/menu/main/home and the LOVD page for *AIP*: https://databases.lovd.nl/shared/genes/AIP. We strongly encourage users to deposit positive results in any of the *AIP* mutation databases. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

The LOVD page for *CDKN1B:* https://databases.lovd.nl/shared/genes/CDKN1B. We strongly encourage users to deposit positive results in the *CDKN1B* database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

COSMIC mutation database

Somatic mutations in *MEN1*, *AIP* and *CDKN1B* in cancer can be found in the COSMIC database at https://cancer.sanger.ac.uk/cosmic.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *AIP* exons 3 and 5 but not exon 4 to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P244-D1 AIP-MEN1-CDKN1B

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*MEN1-AIP* region	*CDKN1B*
64-105	Control fragments – see table in probemix content section for more information			
129 *	Reference probe 18709-L26847	5q31		
136 ¥ «	**MEN1** probe 22342-L02795		**Exon 9**	
142 * ¬	**GPRC5A** probe 22344-L03556			**Downstream**
148	**CDKN1B** probe 18425-L23733			**Exon 2**
154 *	Reference probe 04540-L28278	2q24		
160 « ¬	**BRMS1** probe 04155-L03510		**11q13**	
167 «	**MEN1** probe 13158-L14680		**Exon 4**	
175 «	**AIP** probe 07380-L09559		**Exon 1**	
184 *	Reference probe 10904-L27810	9q34		
191 «	**MEN1** probe 01663-L01242		**Upstream**	
195 «	**MEN1** probe 18765-L24187		**Exon 6**	
202 «	**MEN1** probe 13159-L14681		**Exon 5**	
209 «	**AIP** probe 07383-L07030		**Exon 4**	
216 *	Reference probe 09103-L31536	4q25		
220 «	**MEN1** probe 01664-L01243		**Exon 2**	
229 « ¬	**RELA** probe 01120-L00060		**11q13**	
238 «	**AIP** probe 07381-L07028		**Exon 2**	
247 «	**MEN1** probe 01164-L00720		**Exon 10**	
256 *	Reference probe 08812-L08872	2p13		
266 «	**AIP** probe 07384-L09556		**Exon 5**	
277 *	Reference probe 13393-L28135	6q12		
283 «	**MEN1** probe 01665-L14816		**Exon 3**	
292 «	**AIP** probe 07379-L09558		**Exon 1**	
301 «	**MEN1** probe 01666-L01245		**Exon 7**	
310 ¬	**CCND1** probe 05403-L04809		**11q13**	
319 «	**AIP** probe 07385-L09557		**Exon 6**	
326 *	Reference probe 16275-L18567	19p13		
338 ¥ ¬	**SNX15** probe 01667-L31522		**11q13**	
346 *	Reference probe 04337-L20895	15q21		
355 « ¬	**FAM89B** probe 04157-L03512		**11q13**	
364 «	**AIP** probe 07382-L09069		**Exon 3**	
373 *	Reference probe 04278-L03682	12q12		
382 ¬	**SART1** probe 04159-L03514		**11q13**	
391	**CDKN1B** probe 18426-L23497			**Exon 1**
400 « +	**MEN1** probe 18427-L23498		**Upstream**	
409 *	Reference probe 14839-L30627	1p34		
420 * « ¬	**SF1** probe 22341-L20815		**11q13**	
427 «	**CDKN1B** probe 18429-L23500			**Exon 3**
436 «	**MEN1** probe 18430-L23501		**Exon 8**	
445 *	Reference probe 10709-L11291	6p12		
454 * ¬	**BCL2L14** probe 22345-L02547			**Upstream**
463 *	Reference probe 15970-L18122	18p11		

[a] See section Exon numbering on page 3 for more information.

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
« 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.
¬ 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.
+ 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 on position 242-243. 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.

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. P244-D1 probes arranged according to chromosomal location

Table 2a. *MEN1-AIP* region (11q13)

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
420 « ¬	22341-L20815	*SF1*		GATTCCAGGAAT-GCCTACAGTTAT	27.9 kb
		MEN1	**NM_001370259.2**		
		stop codon	*1892-1894 (exon 10)*		
247 « »	01164-L00720	Exon 10	1791-1792	CGCCATCAAGCT-GCAACTCACGGC	0.7 kb
136 « »	22342-L02795	Exon 9	1326-1327	CGGCATCTGCAA-ATGGGAGGAGGG	0.6 kb
436 «	18430-L23501	Exon 8	1164-1165	CTTTGAAGTAGC-CAATGATGTCAT	0.6 kb
301 « »	01666-L01245	Exon 7	1028-1029	ACCCCTACATGT-ACCTGGCTGGCT	0.7 kb
195 « »	18765-L24187	Exon 6	972-973	CCTCTACCACAA-GGTGGGGGCATC	0.2 kb
202 « »	13159-L14681	Exon 5	867-868	GCTGCTCTATGA-CCTGGGACATCT	0.4 kb
167 « »	13158-L14680	Exon 4	761-762	GTGACCGCAAGA-TGGAGGTGGCGT	0.4 kb
283 « »	01665-L14816	Exon 3	608-609	AGGATCATGCCT-GGGTAGTGTTTG	2.0 kb
220 « »	01664-L01243	Exon 2	201-202	CGTGGAGCATTT-TCTGGCTGTCAA	0.7 kb
		start codon	*62-64 (exon 2)*		
191 « »	01663-L01242	Upstream	131 nt before exon 1	GAGATCCCAGAA-GCCACAGCGCAG	0.4 kb
400 « +	18427-L23498	Upstream	501 nt before exon 1	GCGGAAGTGGGA-AACGAGTGCTGC	216.5 kb
338 ¬	01667-L31522	*SNX15*		CGAAGGATGACT-TCCTGCGGCACT	545.9 kb
355 « ¬	04157-L03512	*FAM89B*		ACAAACACCTGT-GCCAAGACCTGA	88.3 kb
229 « ¬	01120-L00060	*RELA*		AAAGGACTGCCG-GGATGGCTTCTA	305.6 kb
382 ¬	04159-L03514	*SART1*		CCGCAAGAAGGA-GAAGGAGGTAGT	374.9 kb
160 « ¬	04155-L03510	*BRMS1*		CAGAAGAGATGG-AAGCAGAGGGTG	1141.0 kb
		AIP	**NM_003977.4**		
292 «	07379-L09558	Exon 1	105-106	GAGTCCGGAAGT-TGCCGAAAGGGA	0.1 kb
		start codon	*134-136 (exon 1)*		
175 «	07380-L09559	Exon 1	186-187	AAAACGTGTGAT-ACAGGAAGGCCG	3.9 kb
238 «	07381-L07028	Exon 2	320-321	CCATGGAGCTCA-TCATTGGCAAGA	2.3 kb
364 «	07382-L09069	Exon 3	519-520	ACAGATGCGTGA-ACACAGCTCCCT	0.7 kb
209 «	07383-L07030	Exon 4	676-677	GCAGTGCCACTT-ATCCACCAGGAG	0.3 kb
266 «	07384-L09556	Exon 5	840-841	GCTGCTGCTCAA-CTACTGCCAGTG	0.5 kb
319 «	07385-L09557	Exon 6	997-998	CAGGCTGACTTT-GCCAAAGTGCTG	2157.1 kb
		stop codon	*1124-1126 (exon 6)*		
310 ¬	05403-L04809	*CCND1*		TCCGCCCTCCAT-GGTGGCAGCGGG	-

Table 2b. *CDKNB1*

Length (nt)	SALSA MLPA probe	*CDKN1B* exon[a]	Ligation site NM_004064.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
454 ¬	22345-L02547	*BCL2L14*		GTCATGGACAGA-GGTGTCATGGCC	638.3 kb
391	18426-L23497	Exon 1	470-471	GACCCGGGAGAA-AGATGTCAAACG	1.1 kb
		start codon	*473-475 (exon 1)*		
148	18425-L23733	Exon 2	1022-1023	ATGCCGGTTCTG-TGGAGCAGACGC	2.3 kb
		stop codon	*1067-1069 (exon 2)*		
427 «	18429-L23500	Exon 3	1199-1200	CCTGTATAAGCA-CTGAAAAACAAC	187.3 kb
142 ¬	22344-L03556	*GPRC5A*		GTCTGCAAGGTG-CAGGACTCCAAC	-

[a] See section Exon numbering on page 3 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.
» Detects the same sequence as MEN1 probes in SALSA MLPA Probemix P017-D1 MEN1.
¬ 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.
+ 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 on position 242-243. 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.

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.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

- P017 MEN1: Version D1 of P017 contains mostly the same *MEN1* probes and *MEN1* flanking probes as this P244-D1 probemix. P017-D1 is complemented with additional exon 1 and exon 9 probes and the *MEN1* upstream and exon 8 probes have different ligation sites (see also Table 2b). P017 does not contain probes for the *AIP* gene, *CDKN1B* gene and the other genes in the 11q13 region that do not flank *MEN1* directly. Note that P017 is not suited for tumour DNA research.
- P169 Hirschsprung-1: Contains probes for the *RET* gene, associated with MEN2A and MEN2B.
- P177 CASR: Contains probes for the *CASR* gene, associated with familial hypocalciuric hypercalcaemia, a disease with certain similar symptoms as PHPT.
- P466 CDC73: Contains probes for the *CDC73* gene that is associated with hyperparathyroidism-jaw tumour syndrome, a disease with certain similar symptoms as PHPT.

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Selected publications using SALSA MLPA Probemix P244 AIP-MEN1-CDKN1B

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MEN1 copy number variations have also been detected with the P017 MEN1 probemix. See the selected publications listed in the P017 product description for relevant publications.

P244 product history	
Version	*Modification*
D1	Three new flanking probes have been included and the length of two probes was changed. All reference probes were replaced and the total number of reference probes was increased to twelve.
C1	Three probes for the missing *MEN1* exons were included and probes for each exon of the *CDKN1B* gene are included. Seven reference probes were replaced and one extra reference probe was included.
B1	One *MEN1* probe was removed and two new *MEN1* probes were included. Several reference probes were replaced and four extra control fragments at 88-96-100-105 nt were included.
A1	First release.

Implemented changes in the product description
Version D1-03 – 22 April 2025 (04P) - List of *Selected Publications* shortened. - In Table 1, the MEN1-AIP region column has been updated based on MANE exon numbering. In Table 2a, the gene exon and ligation site columns have been updated based on MANE exon numbering. The *Transcript variants* and *Exon numbering* sections have also been edited to reflect this change. Version D1-02 – 03 February 2021 (04P) - Product description rewritten and adapted to a new template. - Intended Purpose was rewritten. - NM sequences for *AIP* and *CDKN1B* genes were updated and the ligation sites were also updated according to these new versions of the NM reference sequences in Table 2. - *References* and *Selected Publications* were curated and new literature was included. - UK has been added to the list of countries in Europe that accept the CE mark.

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

IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.