Product Description SALSA® MLPA® Probemix P225-E1 PTEN

To be used with the MLPA General Protocol.

Version E1. As compared to previous D2 version, all *KLLN* and HhaI digestion control probes have been removed, thus methylation detection is no longer possible in the shared promoter region of *PTEN/KLLN* genes by MS-MLPA with this probemix. In addition, *PTEN* exon 4 probes have been replaced and one probe has been added for exon 2; two *PTEN*-flanking and two *PTENP1* probes have been replaced; ten reference probes have been replaced and one has been added. Nine probes have a modification in length, not in the targeted sequence. For complete product history see page 9.

Catalogue numbers:
- **P225-025R:** SALSA MLPA probemix P225 PTEN, 25 reactions.
- **P225-050R:** SALSA MLPA probemix P225 PTEN, 50 reactions.
- **P225-100R:** SALSA MLPA probemix P225 PTEN, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 use: The SALSA MLPA probemix P225 PTEN is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *PTEN* gene in order to confirm a potential cause and clinical diagnosis for PTEN Hamartoma Tumour Syndrome (PHTS). This product can also be used to determine predisposition to PHTS. PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and Proteus-like syndrome (PLS). This probemix can be used for copy number detection of the *PTEN* pseudogene (*PTENP1*) in a research setting.

This assay is optimised for use with peripheral blood derived genomic DNA. This probemix can be used on tumour tissue to detect deletions and duplications of the *PTEN* and *PTENP1* genes in a research setting. Copy number changes detected by only a single probe always require validation by another method. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Phosphatase and tensin homolog (*PTEN*) is a tumour suppressor gene that is mutated in a large number of cancers at high frequency. Defects in the *PTEN* gene are the main cause of PTEN Hamartoma Tumour Syndrome (PHTS), which is a dominantly inherited cancer predisposition syndrome. PHTS is a spectrum of disorders characterized by multiple hamartomas in several areas of the body. PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome.

Cowden syndrome (CS; OMIM #158350) is inherited in an autosomal dominant manner and comprises 85% of PHTS cases. CS is characterised by hamartomatous polyps of the gastrointestinal tract, mucocutaneous lesions, and by an increased risk of developing breast cancer (with a lifetime risk of 85%), thyroid cancer (with a lifetime risk of 35%), endometrial cancer (with a lifetime risk of approximately 28%), kidney, colon and other cancers. Affected individuals usually present with macrocephaly, trichilemmomas, and papillomatous papules by their late 20s.

Bannayan-Riley-Ruvalcaba syndrome (BRSS) is inherited in an autosomal dominant manner. It is present at birth and is characterized by macrocephaly, intestinal hamartomatous polyposis, lipomas, pigmented macules of the glans penis, intellectual disability (50% of the cases) and development delay. The risk of developing cancer in BRRS patients with a *PTEN* pathogenic variant is similar to patients with CS.

PTEN-related Proteus syndrome (PS) is a highly variable, severe disorder characterized by progressive segmental or patchy overgrowth of diverse tissues of all germ layers, affecting the skeleton, skin, adipose tissue and central nervous systems. PS is associated with tumours, pulmonary complications, and deep vein thrombosis. Proteus-like syndrome describes individuals that do not meet the diagnostic criteria of Proteus syndrome, but share many of the characteristic signs and symptoms associated with this condition.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1488/.

PTENP1 is biologically active as it can regulate cellular levels of PTEN (via binding to mRNAs that target PTEN) and suppress cell growth. It has been shown that *PTENP1* is selectively lost in sporadic colon cancer (Poliseno et al. 2010). Specific *PTENP1* deletion (not PTEN) was also demonstrated in human melanoma (Poliseno et al. 2011). Furthermore, the importance of *PTENP1* as a tumour suppressor has been recently shown in head and neck squamous cell carcinoma (Liu et al. 2017). The *PTENP1* copy number detection is of a great importance in cancer research, however the clinical validity of this gene is not yet fully established.

Gene structure: The *PTEN* gene spans ~108.7 kb on chromosome 10q23.31. The *PTEN* LRG_311 is identical to the NCBI NG_007466 sequence. The LRG sequence is available at http://www.lrg-sequence.org/.

The *PTENP1* gene spans ~3.9 kb on chromosome 9p13.3. The *PTENP1* NCBI sequence is NR_023917.1. No LRG sequence is available for this gene.

Transcript variants: The NM_000314.6 sequence, see https://www.ncbi.nlm.nih.gov/gene/5728, represents the transcript variant 1 of the *PTEN* gene. This sequence is a reference standard in the RefSeqGene project. The *PTEN* transcript variant 1 encodes three isoforms: PTEN-L (or PTENalpha), the longest isoform resulting from an upstream non-AUG (CUG) start codon; and two shorter isoforms resulting from downstream AUG start codons. PTEN, the most abundant isoform, is derived from the 5'-most AUG start codon. The AUG translation start site is located in exon 1 (1032-1034) and the stop codon is located in exon 9 (2241-2243). The *PTEN* transcript variant 2 (NM_001304718.1; see https://www.ncbi.nlm.nih.gov/nuccore/NM_001304718.1) has an additional exon in intron 5 in the 3' coding region.

For the pseudogene *PTENP1* the information was obtain from the NR_023917.1 sequence; see https://www.ncbi.nlm.nih.gov/gene/11191. This long-noncoding RNA (lncRNA) is composed by 1 exon only.

Exon numbering: The exon numbering used in this P225-E1 PTEN product description for the *PTEN* gene is the exon numbering from the RefSeq transcript NM_000314.6, which is identical to the LRG_311 sequence. The exon numbering used for the *PTENP1* gene is the exon numbering from the RefSeq NR_023917.1. The exon numbering and NM or NR sequence used are from 08/2018, but can be changed (by NCBI) after the release of the product description.

Probemix content: The P225-E1 PTEN probemix contains 49 MLPA probes with amplification products between 130 and 496 nt. It contains 22 probes for the *PTEN* gene, at least one probe per exon, ten flanking probes, and two probes for the pseudogene *PTENP1*. In addition, 15 reference probes are included in this probemix. The identity of the genes detected by the reference probes is available in Table 2c and online (www.mlpa.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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 and 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

Probemix P225 not suitable for methylation analysis: From version E1 onwards, P225 probemix is not suitable for methylation analysis since PTEN/KLLN methylation specific probes have been removed. For methylation analysis of this region, MRC-Holland provides the SALSA MLPA probemix ME001, which contains a PTEN/KLLN methylation specific probe for the shared CpG-island for *KLLN* and *PTEN* genes.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel et al. (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 peripheral blood, free from impurities known to affect MLPA reactions. In a research setting, extracted DNA from tumour tissue can also be used. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference 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. Reference samples should be derived from healthy individuals without a history of PTEN Hamartoma Tumour Syndrome (PHTS). More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://www.coriell.org/) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number GM20125 from the Coriell Institute has been tested at MRC-Holland and can be used as a positive control sample to detect a heterozygous duplication of the complete *PTEN* gene. Sample ID number OPM-2 from the Leibniz Institute DSMZ has also been tested at MRC-Holland and can be used as a positive control sample to detect a homozygous exons 3 to 7 deletion in *PTEN* gene (Ge and Rudikoff 2000). The quality of cell lines can change, therefore samples should be validated before use.

Performance characteristics: The expected number of *PTEN* deletions/duplications which can be detected with P225 probemix is approximately 10% for BRRS, 4-12% for CS and unknown for the other syndromes (https://www.ncbi.nlm.nih.gov/books/NBK1488/; (Pilarski et al. 2011)). Analytical performance for the detection of deletions/duplications in the *PTEN* gene is very high and can be considered >99% (based on a 2009-2018 literature review).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) 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 must 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.mlpa.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 of *PTEN* and *PTENP1* specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (homozygous duplication).

The standard deviation of all probes in the reference samples should be <0.10. hen these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients 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 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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 in some cases not result in inactivation of that gene copy.
- Probemix P225 does not cover the additional exon of the transcript variant 2 NM_001304718.1 locating in the intron 5 of *PTEN* gene.
- Copy number changes detected by reference probes and flanking probes are unlikely to have any relation to the hereditary conditions tested for.

<u>P225 specific note:</u>

- If this probemix is used on DNA samples from tumour tissues, reference probes are more prone to have deviating copy number results than in blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct interpretation of the target region.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *PTEN* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P225 PTEN.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.
- When used on tumour DNA (for research use only): 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.

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. SALSA MLPA probemix ME001 contains a PTEN/KLLN methylation specific probe for the shared CpG-island for *KLLN* and *PTEN* genes.

Copy number changes detected by one or 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.

***PTEN* mutation databases:** https://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=PTEN and https://databases.lovd.nl/shared/genes/PTEN. We strongly encourage users to deposit positive results in the Cosmic Database and in the LOVD Database (Leiden Open Variation Database 3.0). 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 (for germline samples only), false positive results due to SNPs and unusual results (e.g., a duplication of *PTEN* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P225-E1 PTEN

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) [a]			
		Reference	*PTEN*	*PTENP1*	Flanking
64-105	Control fragments – see table in probemix content section for more information				
130 *	Reference probe 19616-L26704	4p13			
137 *	Reference probe 03797-L04594	21q22			
142 *	**PTEN probe** 21999-L30837		**Exon 4**		
148 *	Reference probe 14199-L23450	2q13			
155	**PTEN probe** 13690-L15159		**Exon 6**		
162 ¥	**PTEN probe** 07685-L31034		**Exon 8**		
168 * ¬	**SUFU probe** 21051-L31033				10q24.32
173 *	**PTEN probe** 22000-L30838		**Exon 2**		
178	**PTEN probe** 17314-L20922		**Exon 3**		
184 * ¬	**ZNF25 probe** 05760-L06666				10**p**11.1
190 *	Reference probe 10710-L31035	6p12			
196 ¥ ±	**PTEN probe** 06729-L31036		**Exon 2**		
201 ¥	**PTEN probe** 18254-L31140		**Exon 10**		
208 *	**PTEN probe** 22001-L30839		**Exon 4**		
214	**PTEN probe** 07686-L15591		**Exon 9**		
222 ¥	**PTEN probe** 17387-L30897		**Exon 1**		
229 *	Reference probe 00967-L31037	3q12			
239 ¥ ¬	**PCDH15 probe** 08751-L22240				10q21.1
246 *	Reference probe 08715-L30393	9q21			
252 ¬	**CELF2 probe** 17393-L22030				10**p**14
259 ¬	**BMPR1A probe** 19351-L28591				10q23.2
266 *	Reference probe 07391-L30898	12q1			
274 ¥ ¬	**LGI1 probe** 19294-L30901				10q23.33
280 *	**PTENP1 probe** 22040-L30965			**Exon 1**	
285	**PTEN probe** 17390-L14811		**Exon 2**		
292 *	Reference probe 16435-L30904	18q21			
299 ¥ ¬	**RET probe** 18081-L30902				10q11.21
305 ¬	**ANXA7 probe** 18380-L25185				
312 *	Reference probe 13396-L30900	6q12			10q22.2
319	**PTEN probe** 03639-L21321		**Exon 6**		
328	**PTEN probe** 19293-L25664		**Exon 3**		
337 ¥	**PTEN probe** 17396-L31245		**Exon 9**		
344	**PTEN probe** 18694-L24032		**Exon 3**		
352	Reference probe 05273-L25208	2p22			
359 ±	**PTEN probe** 17397-L25715		**Exon 8**		
369 ¥ ¬	**HTRA1 probe** 08602-L30903				10q26.13
379	**PTEN probe** 03638-L24933		**Exon 5**		
391 *	Reference probe 08872-L30905	1p31			
400 *	**PTENP1 probe** 22042-L31246			**Exon 1**	
409	**PTEN probe** 13032-L22244		**Exon 5**		
418 *	Reference probe 11562-L12309	5q31			
427	Reference probe 08839-L22026	2p13			
436	**PTEN probe** 13692-L21061		**Exon 7**		
444	**PTEN probe** 17395-L21062		**Exon 9**		
454 ¬	**ITIH5 probe** 17392-L21057				10**p**14
465	**PTEN probe** 17394-L21385		**Exon 1**		
475	**PTEN probe** 17386-L22174		**Exon 7**		
486	Reference probe 13594-L22376	19p13			
496 *	Reference probe 14894-L31209	15q21			

(a) The exon numbering used in this P225-E1 PTEN product description for the *PTEN* gene is the exon numbering from the RefSeq transcript NM_000314.6, which is identical to the LRG_311 sequence. The exon numbering used for the *PTENP1* gene is the exon numbering from the RefSeq NR_023917.1. The exon numbering and NM or NR sequence used are from 08/2018, but can be changed (by NCBI) after the release of the product description.

* New in version E1 (from lot E1-0518 onwards).

¥ Changed in version E1 (from lot E1-0518 onwards). Small change in length, no change in sequence detected.
¬ 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.
± SNP rs146326040 and rs562164491 (probe 196 nt and 359 nt, respectively) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by the probe.

Table 2. P225 probes arranged according to chromosomal location

Table 2a. *PTEN*

Length (nt)	SALSA MLPA probe	Gene/ *PTEN* Exon[a]	Location/Ligation site[b] NM_000314.6	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
454 ¬	17392-L21057	*ITIH5*	10**p**14	CGAGCAGAGTCA-TCTTGGATGGTG	3.6 Mb
252 ¬	17393-L22030	*CELF2*	10**p**14	TCCCCCGGTCAT-GGTCGGAAAAGG	27.0 Mb
184 ¬	05760-L06666	*ZNF25*	10**p**11.1	ATGTTATTGTGG-AATTCACCAAGG	4.6 Mb
299 ¬	18081-L30902	*RET*	10q11.21	TCCTCTACCTTA-ACCGGAGCCTGG	12.9 Mb
239 ¬	08751-L22240	*PCDH15*	10q21.1	ACAACATGCTGA-TCAAAGGGACTG	19.0 Mb
305 ¬	18380-L25185	*ANXA7*	10q22.2	AGTCCCACCAGG-TGGAGCAGGCTT	13.8 Mb
259 ¬	19351-L28591	*BMPR1A*	10q23.2	TACTTACATGCA-TGTGTTATTAAT	0.9 Mb
		start codon	*1032-1034 (Exon 1)*		
201	18254-L31140	Exon 1	201-200 reverse	GCTGCAGCTTCC-GAGAGGAGAGAA	0.8 kb
222	17387-L30897	Exon 1	967-968	CCTGCAGAAGAA-GCCCCGCCACCA	0.2 kb
465	17394-L21385	Exon 1	5 nt after Exon 1	TTGACCTGTATC-CATTTCTGCGGC	29.5 kb
285	17390-L14811	Exon 2	0 nt before Exon 2	TAAAGTACTCAG-ATATTTATCCAA	0.1 kb
173	22000-L30838	Exon 2	1184-1183 reverse	CTTACTACATCA-TCAATATTGTTC	0.2 kb
196 ±	06729-L31036	Exon 2	217 nt after Exon 2 reverse	TATCACATAAGT-ACCTGATTATGT	31.0 kb
344	18694-L24032	Exon 3	145 nt before Exon 3	GGGGTATTTGTT-GGATTATTTATT	0.2 kb
328	19293-L25664	Exon 3	28 nt after Exon 3	ATTTGTATGCTT-GCAAATATCTTC	0.2 kb
178	17314-L20922	Exon 3	226 nt after Exon 3	TTGATCTGCTTT-AAATGACTTGGC	5.0 kb
142	21999-L30837	Exon 4	282 nt before Exon 4	AGCACCTGAATT-TACAGTACTCTG	2.3 kb
208	22001-L30839	Exon 4	1279-1280	CAAATTTAATTG-CAGAGGTAGGTA	0.1 kb
379 #	03638-L24933	Exon 5	1436-1437	GGTGTAATGATA-TGTGCATATTTA	0.1 kb
409	13032-L22244	Exon 5	7 nt after Exon 5	AAAAGGTAAGTT-ATTTTTTGATGT	18.9 kb
155 #	13690-L15159	Exon 6	1575-1576	ATAGCTACCTGT-TAAAGAATCATC	0.1 kb
319	03639-L21321	Exon 6	1659-1658 reverse	CTTACTGCAAGT-TCCGCCACTGAA	5.7 kb
475 #	17386-L22174	Exon 7	1736-1737	ACACGACGGGAA-GACAAGTTCATG	0.1 kb
436	13692-L21061	Exon 7	4 nt after Exon 7	TAAAAAAGGTTT-GTACTTTACTTT	3.0 kb
359 # ±	17397-L25715	Exon 8	1973-1974	AATGACAAGGAA-TATCTAGTACTT	0.1 kb
162 #	07685-L31034	Exon 8	45 nt after Exon 8	GACTTGTATGTA-TGTGATGTGTGT	4.1 kb
444	17395-L21062	Exon 9	6 nt before Exon 9	TAAATTTTCTTT-CTCTAGGTGAAG	0.3 kb
337 #	17396-L31245	Exon 9	2357-2356 reverse	AGAGAATTGTTC-CTATAACTGGTA	0.8 kb
214 #	07686-L15591	Exon 9	3189-3188 reverse	ACAGCATCTGAA-TTTTAGCACTGG	5.8 Mb
		stop codon	*2241-2243 (Exon 9)*		
274 ¬	19294-L30901	*LGI1*	10q23.33	CTGAAATGGCTA-GTGGAATGGCTT	8.8 Mb
168 ¬	21051-L31033	*SUFU*	10q24.32	GAGGCCTGGTGA-GAAATGTGTGAT	19.9 Mb
369 ¬	08602-L30903	*HTRA1*	10q26.13	AATTGTTTCGCA-AGTAAAGAGAGC	

(a) The exon numbering used in this P225-E1 PTEN product description for the *PTEN* gene is the exon numbering from the RefSeq transcript NM_000314.6, which is identical to the LRG_311 sequence. The exon numbering used for the *PTENP1* gene is the exon numbering from the RefSeq NR_023917.1. The exon numbering and NM or NR sequence used are from 08/2018, but can be changed (by NCBI) after the release of the product description.

(b) Ligation sites of the P225 PTEN MLPA probes are indicated according to RefSeq sequence NM_000314.6 containing 9 exons.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

¬ 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.

± SNP rs146326040 and rs562164491 (probe 196 nt and 359 nt, respectively) could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by the probe.

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.

Table 2b. *PTENP1*

Length (nt)	SALSA MLPA probe	*PTENP1* exon[a]	Ligation site[b] NR_023917.1	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
280 #	22040-L30965	Exon 1	2423-2424	AGTTTGCAGTTA-GCTAAGAGAAGT	0.3 kb
400 #	22042-L31246	Exon 1	2109-2110	TTTCTCTTTTCG-TGACCAATCTTG	

(a) The exon numbering used in this P225-E1 PTEN product description for the *PTEN* gene is the exon numbering from the RefSeq transcript NM_000314.6, which is identical to the LRG_311 sequence. The exon numbering used for the *PTENP1* gene is the exon numbering from the RefSeq NR_023917.1. The exon numbering and NM or NR sequence used are from 08/2018, but can be changed (by NCBI) after the release of the product description.

(b) Ligation sites of the P225 PTEN MLPA probes are indicated according RefSeq sequence NR_023917.1 containing 1 exon.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

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.

Table 2c. Reference probes

Length (nt)	SALSA MLPA probe	Gene	Location (hg 18)	Partial sequence[c] (24 nt adjacent to ligation site)
391	08872-L30905	*LEPR*	1p31	AGCACATACTGT-TACGGTTCTGGC
427	08839-L22026	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT
352	05273-L25208	*SPAST*	2p22	CGAGCCACAGCA-AAAAGAGCCCTC
148	14199-L23450	*EDAR*	2q13	GAGAGTTCTGTG-GGTGGAGAGAAG
229	00967-L31037	*SENP7*	3q12	CAGTCATTTGTT-TTCCATGGTTAG
130	19616-L26704	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG
418	11562-L12309	*MYOT*	5q31	TCAGGATCTCAA-CAAGGAAGAGCA
190	10710-L31035	*PKHD1*	6p12	GGTTCCTGCTCT-TTCCAGTACCTC
312	13396-L30900	*EYS*	6q12	AAGGTTTGATGT-ACTCACCTACAA
246	08715-L30393	*PCSK5*	9q21	AAGCTGAGACCT-AGTTCCAGAGGG
266	07391-L30898	*COL2A1*	12q13	TGAACCTGGTGA-ACCTGGTGTCTC
496	14894-L31209	*SPG11*	15q21	GGACAATTCGCT-TTGGCCAGGAGG
292	16435-L30904	*MYO5B*	18q21	GAACAGCTCAAC-AACCAAATCCTG
486	13594-L22376	*CACNA1A*	19p13	ACTGGAGGAATG-GCAGCCCCTGGT
137	03797-L04594	*KCNJ6*	21q22	CTCGAAGCTCCT-ACATCACCAGTG

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

ME001 Tumour suppressor mix 1	Tumour suppressor genes; including *KLLN/PTEN* shared promoter region.
P067 PTCH1	Gorlin syndrome; gene included: *PTCH1*.
P081/P082 NF1 mix 1 & mix 2	Neurofibromatosis type 1; gene included: *NF1.*
P101 STK11	Peutz-Jeghers syndrome; gene included: *STK11.*
P158 JPS	Juvenile Polyposis; genes included: *BMPR1A*, *SMAD4* and *PTEN.*
P256 FLCN	Birt-Hogg-Dube syndrome; gene included: *FLCN.*
P472 SUFU	Familial Medulloblastoma and Meningioma; gene included: *SUFU.*

References

- Ge NL et al. (2000). Expression of PTEN in PTEN-deficient multiple myeloma cells abolishes tumor growth in vivo. *Oncogene.* 19:4091-5.
- Hömig-Hölzel C et al. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Liu J et al. (2017). Decreased expression of pseudogene PTENP1 promotes malignant behaviours and is associated with the poor survival of patients with HNSCC. *Sci Rep.* 7:41179.
- Pilarski R et al. (2011). Predicting PTEN mutations: an evaluation of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome clinical features. *J Med Genet.* 48:505-12.
- Poliseno L et al. (2010). A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature.* 465:1033-8.
- Poliseno L et al. (2011). Deletion of PTENP1 pseudogene in human melanoma. *J Invest Dermatol.* 131:2497-2500.
- 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 P225 PTEN

- Ge NL et al. (2000). Expression of PTEN in PTEN-deficient multiple myeloma cells abolishes tumor growth in vivo. *Oncogene.* 19:4091-4095.
- Liu J et al. (2017). Decreased expression of pseudogene PTENP1 promotes malignant behaviours and is associated with the poor survival of patients with HNSCC. *Sci Rep.* 7:41179.
- Pilarski R et al. (2011). Predicting PTEN mutations: an evaluation of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome clinical features. *J Med Genet.* 48:505-512.
- Poliseno L et al. (2010). A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature.* 465:1033-1038.
- Poliseno L et al. (2011). Deletion of PTENP1 pseudogene in human melanoma. *J Invest Dermatol.* 131:2497-2500.

P225 Product history	
Version	*Modification*
E1	All *KLLN* and HhaI digestion control probes have been removed, thus methylation detection is no longer possible in the shared promoter region of *PTEN/KLLN* genes by MS-MLPA with this probemix. In addition, *PTEN* exon 4 probes have been replaced and one probe has been added for exon 2; two *PTEN*-flanking and two *PTENP1* probes have been replaced; ten reference probes have been replaced and one has been added. Nine probes have a modification in length, not in the targeted sequence.
D2	One probe has a small change in length but no change in the sequence detected.

D1	One probe for *PTEN* exon 3 and one flanking probe for *PTEN* have been added and four flanking probes have been replaced. An HhaI digestion control probe has been included and several reference probes have been replaced.
C1	Several new target and reference probes have been added. P225 may also be used to detect methylation of *PTEN* and *KLLN*.
B3	The 88 and 96 nt control fragments have been replaced (QDX2).
B2	The number of PTEN probes has been increased to 25.
B1	Eight new PTEN probes have been added and three variable PTEN probes have been removed. In order to facilitate analysis of tumour DNA, several chromosome 10 probes have been included.
A1	Extra control fragments at 88, 96, 100 and 105 nt have been added.
A0	First release.

Implemented changes in the product description

Version E1-01 – 19 October 2018 (04)
- Product description restructured and adapted to a new template.
- Several references removed and added.
- Warning added to Table 1 and 2a for SNPs that could influence probe signal: 196 nt probe 06729-L31036, and 359 nt probe 17397-L25715.
- Warning added to Table 2a and 2b for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 23 – 15 February 2018 (T08)
- Warning added in Tables 1 and 2 concerning a single nucleotide difference between the PTEN gene and the PTEN pseudogene (PTENP1) for several probes.
- Warning added in Tables 1 and 2 for a frequent SNP (rs12000677) in probe 17308-L25183

Version 22 – 9 September 2017 (T08)
- Warning added in Table 1, 172 nt probe 17388-L08261, 227 nt probe 17387-L26030, 364 nt probe 18110-L25928, and 465 nt probe 17394-L21385.
- Small changes of S0750-L21493 probe length in Table 1 and 2 in order to better reflect the true length of the amplification product.

Version 21 – 21 March 2017 (T08)
- Various minor textual changes.

Version 20 – 02 December 2016 (T08)
- Warning regarding HhaI enzymes that are resistant to heat inactivation added under Methylation-specific MLPA section.
- Various minor textual changes.

Version 19 – 17 June 2016 (T08)
- New references added on page 2.
- Warning added in Table 1 and Table 2, 208 nt probe 17391-L21278.

Version 18 – 23 June 2015 (T07)
- Small changes of the probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 17 – 29 May 2015 (T07)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new figures included).
- Small changes of the probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- New references added on page 2.
- Various minor textual changes.
- New Figure 3 added to pinpoint peak at 97 nt in no DNA reaction.

Version 16 – 20 November 2013 (T05)
- Product description adapted to a new lot (lot number added, changes in Table 1 and Table 2, new figures included).
- References added on page 2.

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

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