Instructions for Use
SALSA® MLPA® Probemix P225 PTEN

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 P225 PTEN product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P225 PTEN**
Version	E1
Catalogue numbers	P225-025R (25 reactions) P225-050R (50 reactions) P225-100R (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 D2, 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* 8*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.

1. Intended Purpose

The SALSA MLPA Probemix P225 PTEN is an in vitro diagnostic (IVD)[1] or research use only (RUO) semiquantitative assay[2] for the detection of deletions or duplications in *PTEN* in genomic DNA isolated from human peripheral whole blood specimens. P225 PTEN is intended to confirm a potential cause for and clinical diagnosis of PTEN Hamartoma Tumour Syndrome (PHTS) and for molecular genetic testing of at-risk family members. PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS), and Proteus-like syndrome (PLS). This probemix can also be used for the detection of deletions or duplications in the *PTEN* pseudogene (*PTENP1*) in a research setting.

Copy number variations (CNVs) detected with P225 PTEN 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 *PTEN* 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, parental evaluation, 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. 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.

[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 PTEN Hamartoma Tumour Syndrome (PHTS).	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*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 (*PTEN*)

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.

These final ratios are only valid for germline testing. In a research setting, when using DNA samples isolated from tumour tissues, the criterium for the FR of each individual reference probe in patient samples is not applicable.

6. Performance Characteristics

Changes in the copy number of *PTEN* account for 3% of CS and 11% of BRRS cases, according to GeneReviews (https://www.ncbi.nlm.nih.gov/books/NBK1488/). It is unknown how many PLS and PS cases can be explained by copy number changes in *PTEN*, however, the association between *PTEN* mutations and PLS and PS is well established (Eng 2003, Loffeld et al. 2006, Orloff and Eng 2008, Smith et al. 2002, Zhou et al. 2001). The analytical performance for the detection of deletions/duplications in the *PTEN* gene is very high and can be considered >99% (based on a 2009-2024 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/normalization.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
9p13.3	*PTENP1*	Exon 1	0.3 kb	280	22040-L30965	#
9p13.3	*PTENP1*	Exon 1		400	22042-L31246	#
10p14	*ITIH5*		3.6 **M**b	454	17392-L21057	¬
10p14	*CELF2*		27.0 **M**b	252	17393-L22030	¬
10p11.21	*ZNF25*		4.6 **M**b	184	05760-L06666	¬ Δ
10q11.21	*RET*		12.9 **M**b	299	18081-L30902	¬
10q21.1	*PCDH15*		19.0 **M**b	238	08751-L22240	¬
10q22.2	*ANXA7*		13.8 **M**b	305	18380-L25185	¬
10q23.2	*BMPR1A*		0.9 **M**b	259	19351-L28591	¬
10q23.31	*PTEN*	Exon 1	0.8 kb	201	18254-L31140	
10q23.31	*PTEN*	Exon 1	0.2 kb	222	17387-L30897	
10q23.31	*PTEN*	Exon 1	29.5 kb	465	17394-L21385	
10q23.31	*PTEN*	Exon 2	0.1 kb	286	17390-L14811	
10q23.31	*PTEN*	Exon 2	0.2 kb	173	22000-L30838	
10q23.31	*PTEN*	Intron 2 (Exon 2)	31.0 kb	195	06729-L31036	Ø
10q23.31	*PTEN*	Intron 2 (Exon 3)	0.2 kb	344	18694-L24032	Ø
10q23.31	*PTEN*	Exon 3	0.2 kb	328	19293-L25664	+
10q23.31	*PTEN*	Intron 3 (Exon 3)	5.0 kb	178	17314-L20922	Ø
10q23.31	*PTEN*	Intron 3 (Exon 4)	0.3 kb	142	21999-L30837	Ø
10q23.31	*PTEN*	Exon 4	2.1 kb	208	22001-L30839	
10q23.31	*PTEN*	Exon 5	0.1 kb	379	03638-L24933	#
10q23.31	*PTEN*	Exon 5	18.9 kb	409	13032-L22244	
10q23.31	*PTEN*	Exon 6	0.1 kb	155	13690-L15159	#
10q23.31	*PTEN*	Exon 6	5.7 kb	319	03639-L21321	
10q23.31	*PTEN*	Exon 7	0.1 kb	475	17386-L22174	#
10q23.31	*PTEN*	Exon 7	3.0 kb	436	13692-L21061	
10q23.31	*PTEN*	Exon 8	0.1 kb	359	17397-L25715	#
10q23.31	*PTEN*	Exon 8	4.1 kb	162	07685-L31034	# Δ +
10q23.31	*PTEN*	Exon 9	0.3 kb	444	17395-L21062	
10q23.31	*PTEN*	Exon 9	0.8 kb	337	17396-L31245	#
10q23.31	*PTEN*	Exon 9	5.8 **M**b	214	07686-L15591	#
10q23.33	*LGI1*		8.8 **M**b	272	19294-L30901	¬
10q24.32	*SUFU*		19.9 **M**b	168	21051-L31033	¬
10q26.13	*HTRA1*			368	08602-L30903	¬
1p	Reference			391	08872-L30905	
2p	Reference			353	05273-L25208	
2p	Reference			427	08839-L22026	
2q	Reference			148	14199-L23450	
3q	Reference			229	00967-L31037	
4p	Reference			130	19616-L26704	
5q	Reference			418	11562-L12309	
6p	Reference			190	10710-L31035	
6q	Reference			312	13396-L30900	
9q	Reference			246	08715-L30393	
12q	Reference			266	07391-L30898	
15q	Reference			496	14894-L31209	
18q	Reference			292	16435-L30904	
19p	Reference			485	13594-L22376	
21q	Reference			280	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 *PTEN* 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.
Annotations of several probes with targets at the edge of or slightly outside the coding region, were 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.

Δ These probes may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

\+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

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

The specificity of these probes rely on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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. In a research setting, when using DNA samples isolated from tumour tissues, reference probes are more prone to have deviating copy number results as compared to 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 copy number interpretation on the target region.
6. P225 PTEN does not cover the additional exon located in *PTEN* intron 5, part of transcript variant 2 (NM_001304718.2).
7. To our knowledge, only heterozygous *PTEN* deletions are expected in the germline, whereas both, homozygous and heterozygous *PTEN* deletions are expected in somatic tissue.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. 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.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Eng C et al. (2003). PTEN: one gene, many syndromes. Hum Mutat. 22(3):183-98
2. Loffeld A et al. (2006). Epidermal naevus in Proteus syndrome showing loss of heterozygosity for an inherited PTEN mutation. Br J Dermatol. 154(6):1194-8.
3. Orloff MS and Eng C. (2008). Genetic and phenotypic heterogeneity in the PTEN hamartoma tumour syndrome. Oncogene. 27(41):5387-97.
4. Smith JM et al. (2002). Germline mutation of the tumour suppressor PTEN in Proteus syndrome. J Med Genet. 39(12):937-40.
5. Zhou X et al. (2001). Association of germline mutation in the PTEN tumour suppressor gene and Proteus and Proteus-like syndromes. Lancet. 358(9277):210-1.

Implemented changes in the product description
Version E1-07 – 26 February 2025 *(03S)* - Updated to a new template. - SNVs rs146326040 and rs562164491 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Salt warning has been removed from *SUFU* probe 21051-L31033. - Warning for a ligation site >20 nt away from the nearest exon added to *PTEN* probes 19293-L25664 and 07685-L31034. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Warning for probes targeting sequences outside of the known coding region included for probes 06729-L31036, 18694-L24032, 17314-L20922, and 21999-L30837. - Adjusted the distance to next probe for probes 21999-L30837 and 22001-L30839.