Instructions for Use
SALSA® MLPA® Probemix P008 PMS2

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit and SALSA® Reference Selection DNA SD082, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P008 PMS2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P008 PMS2**
Version	C1
Catalogue numbers	P008-025R (25 reactions) P008-050R (50 reactions) P008-100R (100 reactions)
Basic UDI-DI	872021148P0085L
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Reference Selection DNA SD082	SD082

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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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As compared to version B2 (sold until May 2014), the *PMS2* exon 3 and 4 probes and one reference probe have been replaced by two new exon 3 probes and one new exon 4 probe. The new probes generate almost no signal on the *PMS2* pseudogenes. One of the exon 2 probes was removed.

1. Intended Purpose

The SALSA MLPA Probemix P008 PMS2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *PMS2* gene in genomic DNA isolated from human peripheral whole blood specimens. P008 PMS2 is intended to confirm a potential cause for and clinical diagnosis of Lynch syndrome (deletions or duplications in *PMS2,* in the context of a monoallelic variant) or constitutional mismatch repair deficiency syndrome (deletions in *PMS2*, in the context of biallelic pathogenic variants) and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P008 PMS2 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 *PMS2* 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 of the *PMS2* gene. To determine whether a CNV of exons 12-15 is present in *PMS2* **or** *PMS2CL*, MLPA should always be combined with other methods, for example gene-specific long-range PCR and sequencing of the amplification products.

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, 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 copy number of 2 for each of the ten SNV probe targets, and a normal copy number with a ≤0.10 standard deviation for all other probes. More information can be found on the P008 PMS2 product page on our website. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of hereditary predisposition to cancer. • **Selected using SALSA Reference Selection DNA SD082.** Refer to Reference Selection DNA below for more information.
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination
Reference Selection DNA	• SALSA Reference Selection DNA SD082, available from MRC Holland. • Use SD082 to identify suitable reference samples. • **SD082 should never be used as a reference sample.** • More information can be found on the P008 PMS2 product page on our website.
Positive Control Samples (Preferably)	• Provided by user, or • Available from third parties

*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

The tables present in this section illustrate the relationship between final 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 an SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found. Due to the presence of pseudogene *PMS2CL*, result interpretation for the *PMS2* region is complicated. Please refer to the PMS2 product page on our website for more information.

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 (PMS2-specific[3])

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

[3] See column *Target* in Table named *Content – Probe Details Sorted by Chromosomal Position*.

Typical Results of Probes Targeting Four Copies (combined PMS2&PMS2CL[4])

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion of *PMS2* **AND** *PMS2CL*
~ 0.25	1	Homozygous deletion of *PMS2* **OR** *PMS2CL* **AND** Heterozygous deletion of *PMS2* **OR** *PMS2CL*
~ 0.5	2	Heterozygous deletion of *PMS2* **AND** *PMS2CL* **OR** Homozygous deletion of *PMS2* **OR** *PMS2CL*
~ 0.75	3	Heterozygous deletion of *PMS2* **OR** *PMS2CL* **OR** Homozygous deletion of *PMS2* **OR** *PMS2CL* **AND** Heterozygous duplication of *PMS2* **OR** *PMS2CL*
~ 1	**4**	**Normal**
~ 1.25	5	Heterozygous duplication of *PMS2* **OR** *PMS2CL* **OR** Homozygous duplication of *PMS2* **OR** *PMS2CL* **AND** Heterozygous deletion of *PMS2* **OR** *PMS2CL*
~ 1.5	6	Various combinations of CNVs possible
~ 1.75	7	
~ 2	8	

[4] See probes with ∫ warning in Table named *Content – Probe Details Sorted by Chromosomal Position*.

Typical Results of PMS2/PMS2CL SNV Probe Pairs[5]

Final Ratio (FR)	Number of copies with specific SNV (SNV 1 or SNV 2 from the pair) - present in *PMS2* or *PMS2CL*
0	0
0.40 – 0.65	1
0.80 – 1.20	2
1.30 – 1.65	3
1.75 – 2.15	4
All other values	Ambiguous

[5] See probes with ~ warning in Table named *Content – Probe Details Sorted by Chromosomal Position*.

The table titled *Typical Results of PMS2/PMS2CL SNV Probe Pairs* can be used to determine the copy number of a specific SNV targeted by an SNV probe based on its final ratio. The table titled *Interpretation of Typical Results from PMS2/PMS2CL SNV Probe Pairs* present on the next page can be used to interpret the result of pairs of probes targeting different SNVs of the same rsID.

Interpretation of Typical Results from PMS2/PMS2CL SNV Probe Pairs

Number of copies with specific SNV (SNV 1 or SNV 2 from the pair) **- present in *PMS2* or *PMS2CL***		**Total number of copies with either SNV - present in *PMS2* or *PMS2CL***	**Most Commonly Encountered Underlying Genotype**[6]
SNV 1	**SNV 2**	**SNV 1 + SNV 2**	
0	0	0	Homozygous deletion of *PMS2* **AND** *PMS2CL*
0	1	1	Various combinations of CNVs possible
1	0		
0	2	2	Heterozygous deletion of *PMS2* **AND** *PMS2CL* OR Homozygous deletion of *PMS2* **OR** *PMS2CL*
1	1		
2	0		
0	3	3	Heterozygous deletion of *PMS2* **OR** *PMS2CL*
1	2		
2	1		
3	0		
0	**4**	**4**	**Normal**
1	**3**		
2	**2**		
3	**1**		
4	**0**		
1	4	5	Heterozygous duplication of *PMS2* **OR** *PMS2CL*
2	3		
3	2		
4	1		
2	4	6	Various combinations of CNVs possible
3	3		
4	2		
3	4	7	
4	3		
4	4	8	

[6] The total number of copies of *PMS2*/*PMS2CL* with each SNV can stem from a different genotype than the examples provided in this table. The situations displayed in the table are those most commonly encountered for germline DNA. Other genotypic descriptions can occur.

An extensive explanation on reference sample selection and data interpretation, including examples of MLPA results obtained with this probemix can be found on the P008 PMS2 product page.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used.

Cut-off values for copy number determination were verified with P008 PMS2 in 46 samples from healthy individuals with normal copy number and two samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested, with the exception of three ambiguous out of 1716 measurements.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance.

These lower and higher limits of detection were verified using P008 PMS2 on two samples with known CNVs and on one sample without any mutation. Expected results were obtained using both the lower and upper input amount of DNA, with the exception of five ambiguous out of 396 measurements. Such data would only lead to delayed results, as the assay may need to be repeated. False positives or negatives would not ensue.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using P008 PMS2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs and one sample without any mutation. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Expected FR for 289/297 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 293/297 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 290/297 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 292/297 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 252/297 measurements</td></tr>
</table>
* Results are summarised for 33 PMS2/PMS2CL probes across all three samples tested.

Most substances did not interfere with copy number determination as compared to controls, while an effect (some ambiguous measurement and some in the wrong CN category) on the FRs was observed for a low number of probes with EDTA. Hemoglobin had the largest effect on the results.

Coffalyser.Net issued warnings for samples in which deviation was observed.

To minimise variability across samples, all samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible.</td></tr>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, two previously genotyped samples were tested using P008 PMS2 and found to have the expected results. Assay precision was tested by repeatedly testing three samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 1462/1485 measurements, leading to a precision of 98%.</td></tr>
<tr><td>Clinical validity*</td><td>1-13.75% of LS is caused by large deletions or duplications in PMS2 (GeneReviews).

~12% of CMMRD is caused by large deletions in PMS2 (homozygous or compound heterozygous) (Herkert, Niessen et al. 2011, Bodo, Colas et al. 2015).

*(Based on a 2005-2025 literature review)</td></tr>
</table>

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	rsID[7]	SNV	Length (nt)	Probe number	Warnings
7p22.1	*PMS2/PMS2CL*	Exon 15	0.2 kb	rs267608145	TTT	356	14460-L16180	~
					TT	349	14460-L04046	~
7p22.1	*PMS2&PMS2CL*	Exon 15	4.0 kb			409	01189-L00750	∫
7p22.1	*PMS2&PMS2CL*	Exon 14	0.1 kb			190	15768-L18167	+ ∫
7p22.1	*PMS2&PMS2CL*	Exon 14	0.1 kb			382	15293-L17051	∫
7p22.1	*PMS2/PMS2CL*	Exon 14	0.9 kb	rs17420802	C	208	14458-L16177	~
					T	202	14458-L16176	~
7p22.1	*PMS2/PMS2CL*	Exon 13	0.1 kb	rs1805325	G	220	14456-L16512	~ δ
					A	214	14456-L16511	~ δ
7p22.1	*PMS2&PMS2CL*	Exon 13	3.0 kb			261	15767-L17448	∫
7p22.1	*PMS2/PMS2CL*	Intron 12	1.2 kb	rs35748755	GTA	238	14455-L16168	Ø ~ ∑
					ACC	244	14455-L16169	Ø ~ Ω
7p22.1	*PMS2&PMS2CL*	Exon 12	4.0 kb			283	15769-L17786	∫
7p22.1	*PMS2*	Exon 11	0.4 kb			364	14451-L16163	# ¤
7p22.1	*PMS2/PMS2CL*	Exon 11	0.1 kb	rs63750685	C	171	14453-L16165	~ Ω
					G	165	14453-L16164	~ ∑
7p22.1	*PMS2*	Exon 11	2.4 kb			133	14452-L00900	# ¤
7p22.1	*PMS2*	Exon 10	0.1 kb			319	01184-L00745	
7p22.1	*PMS2*	Exon 10	2.1 kb			463	14450-L16162	
7p22.1	*PMS2*	Exon 9	0.1 kb			140	14448-L16160	#
7p22.1	*PMS2*	Exon 9	3.5 kb			445	14449-L16622	# +
7p22.1	*PMS2*	Exon 8	1.8 kb			299	01182-L16159	
7p22.1	*PMS2*	Exon 7	1.8 kb			276	01181-L16158	
7p22.1	*PMS2*	Exon 6	0.1 kb			250	01180-L16157	
7p22.1	*PMS2*	Exon 6	3.3 kb			436	14447-L16623	
7p22.1	*PMS2*	Exon 5	0.1 kb			454	14446-L16621	# Ᵽ
7p22.1	*PMS2*	Exon 5	1.0 kb			232	14445-L16154	# Ᵽ
7p22.1	*PMS2*	Exon 4	0.3 kb			418	19906-L26893	# +
7p22.1	*PMS2*	Exon 3	0.3 kb			310	19910-L26895	# +
7p22.1	*PMS2*	Intron 2 (Exon 3)	1.7 kb			390	19915-L26898	# Ø
7p22.1	*PMS2*	Exon 2	0.1 kb			184	01176-L16620	#
7p22.1	*PMS2*	Exon 2	3.1 kb			400	14441-L16150	#
7p22.1	*PMS2*	Exon 1	0.2 kb			146	07935-L16148	# +
7p22.1	*PMS2*	Upstream (Exon 1)				338	07934-L16147	# Ø
2p	Reference					292	11087-L11770	
3q	Reference					328	08543-L08544	
3q	Reference					268	19040-L09299	
5q	Reference					128	00797-L00093	
6p	Reference					154	02417-L04306	
7p	Reference					177	04359-L03779	
8q	Reference					472	15978-L18133	
10p	Reference					483	08480-L08491	
11p	Reference					427	06029-L05485	
11p	Reference					226	07083-L06712	
14q	Reference					196	07510-L07172	
17q	Reference					373	02528-L01959	
17q	Reference					160	08583-L08584	

[7] The provided rsIDs refer to variants in *PMS2*. These rsIDs are used as a point of reference, but please note that either SNV can be present in *PMS2* or *PMS2CL*.

For PMS2/PMS2CL SNV probe pairs (shaded boxes), the nucleotide(s) targeted by each probe is given. Targeted SNVs are given as the nucleotides in the + strand of the reference genome. The notation of these probe pairs differs from that of the previous version of the product description.

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 *PMS2* exon numbers are derived from MANE project and are based on MANE Select transcript. For ease of interpretation, exon numbering throughout this document refers to the exons of *PMS2*, also when referring to the corresponding homologous exon present in *PMS2CL*. 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

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 relies 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.
+	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.
∫	These probes detect *PMS2* **and** its pseudogene *PMS2CL*. See 'Typical Results of Probes Targeting Four Copies (combined *PMS2&PMS2CL*)' in the Interpretation of Results section of this product description for information on interpretation of these probes.
~	Shaded boxes. These ten probes each detect a SNV which is present in two allelic forms. Due to frequent gene conversion events, these probes cannot confidently be assigned to *PMS2* or its pseudogene *PMS2CL* (in particular the probes for *PMS2* exons 13, 14 and 15, or corresponding exons in *PMS2CL*). When the combined copy number of the two SNVs is 4, a deletion or duplication is unlikely. The PMS2/PMS2CL SNV probe pairs for *PMS2* exon 11 (165 nt and 171 nt) and intron 12 (238 nt and 244 nt) (or corresponding exons/introns in *PMS2CL*) are more stable. Please note that gene conversions may also occur for these SNV probe pairs, and that the obtained results should always be confirmed. See *Typical Results of PMS2/PMS2CL SNV Probe Pairs* in the Interpretation of Results section of this product description for information on interpretation of these probes.
∑	In most cases, the 165 nt and 238 nt probes reflect the copy number of *PMS2*. A limited amount of data is available on the current status of divergence between *PMS2* and *PMS2CL*.
Ω	In most cases, the 171 nt and 244 nt probes reflect the copy number of *PMS2CL*.
¤	Note that gene conversion may occur between *PMS2* and *PMS2CL* in the region targeted by these probes. In case of an apparent aberration of only these probes, further investigation should take place.
₽	The two probes for *PMS2* exon 5 generate a (limited) signal on one of the many *PMS2* pseudogenes. On a sample with a homozygous deletion of *PMS2*, the 232 nt exon 5 probe generated a signal of ~10% of the normal signal, while the 454 nt exon 5 probe generated a signal of ~20% of the normal signal. On samples with a heterozygous deletion of *PMS2* exon 5, we expect a signal of ~60-70% of the normal signal for these probes. When the ligation reaction is performed at room temperature, the 454 nt exon 5 probe is more prone to bind to homologous sequences. A deletion or duplication of *PMS2* exon 5 should particularly be further investigated if both exon 5 probes show the same result.
δ	Due to the high sequence similarity between the probes in this PMS2/PMS2CL SNV probe pair, in samples where *PMS2* and *PMS2CL* both present the same variant (**either** the rs1805325 A **or** the rs1805325 G variant), a small background signal can be seen for the SNV probe targeting the other (absent) variant.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Before testing patient samples, unaffected samples must be screened to identify suitable reference samples for accurate data analysis. Selecting suitable reference samples for the P008 PMS2 probemix is complicated due to the presence of ten PMS2/PMS2CL SNV probes in this probemix. Suitable reference samples have two copies for each of the ten SNV probe targets. In our experience, only 25% of unaffected samples meet this requirement. SALSA Reference Selection DNA SD082 (provided with this probemix) is an aid that can be used to select suitable reference samples from your own sample collection. SD082 should not be used as a reference sample in subsequent experiments. More information on the selection of suitable reference samples can be found on the P008 PMS2 product page on our website.
6. Several PMS2 probes depend for their specificity on a single nucleotide difference between the *PMS2* gene and its pseudogenes. As a result, an apparent duplication of those probes can be the result of a clinically non-significant single nucleotide sequence change in one of the pseudogenes.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. Detection of abnormal copy numbers of *PMS2* exons is complicated due to the existence of many pseudogenes. No reliable sequence differences exist between exons 12-15 of *PMS2* and the corresponding exons in the *PMS2CL* pseudogene (van der Klift et al. 2010). For this reason, five probes are included that detect sequences present in exons 12-15 of both *PMS2* and the corresponding exons in *PMS2CL*. As these probes detect both genes, a healthy individual has four copies per cell. Together with the probes targeting exons 1-11 of *PMS2*, the analysis of these probes should exclude *PMS2* CNVs in the great majority of samples tested. In case one or more of these five probes do indicate a copy number change in the *PMS2* exon 12-15 region (or corresponding region in *PMS2CL*), it should be determined whether the copy number change is in *PMS2* or in *PMS2CL*. To facilitate this, ten probes are included that detect the copy number of both allelic forms of five SNVs in exons 11-15 of *PMS2* or the corresponding exons in *PMS2CL*. The distribution of these SNVs among *PMS2* and *PMS2CL* varies. Analysis of these SNV-specific probes, complemented with gene-specific long-range PCR and (next generation) sequencing analysis, can give further indications on whether an apparent CNV is in exons 12-15 of the *PMS2* gene or in the corresponding exons of *PMS2CL*

(Li et al. 2015, Vaughn et al. 2011). Allocation of the CNV to either *PMS2* or *PMS2CL* will not be possible when all SNVs of *PMS2* and *PMS2CL* share the same variant targeted by the PMS2/PMS2CL SNV probes (Vaughn et al. 2011). In such situations, family studies will be required to determine whether the CNV is present in *PMS2* or *PMS2CL*. More information on result interpretation can be found on the P008 PMS2 product page on our website.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Dong L *et al.* (2020). Distinct clinical phenotype and genetic testing strategy for Lynch syndrome in China based on a large colorectal cancer cohort. *Int J Cancer*. 146:3077-3086.
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Implemented changes in the product description

Version C1-08 – 8 May 2026 (03S)
- Product description adapted to new template.
- Intended purpose adjusted: specification that this is a manual assay; specifications of CNVs related to LS (deletions or duplications in *PMS2,* in the context of a monoallelic variant) and CMMRD (deletions in *PMS2*, in the context of biallelic pathogenic variants) updated; removal of *PMS2CL* in the context of assay function; removal of reference sample identification specification as information present elsewhere.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warning added for PMS2&PMS2CL probe 15768-L18167, and PMS2-specific probes 14449-L16622, 19906-L26893, 19910-L26895 and 07935-L16148 having a ligation site more than 20 nt away from the nearest exon.
- Warning for gene conversion has been added for PMS2-specific probes 14451-L16163 and 14452-L00900.
- Warning added for a background signal in one of the PMS2/PMS2CL SNV probe pairs.
- Restructured warning symbols for PMS2/PMS2CL SNV probe pairs in the content table.
- SNVs targeted by SNV-specific probes mentioned in the table updated to reference the nucleotide as present in the forward strand.
- The Appendix has been removed in favour of information being placed on the P008 product page.
- Performance characteristics updated with data from analytical performance experiments.
- Reference to the SSP added.
- CE mark with notified body number added.
- Probemix is now IVDR certified.