Instructions for Use

SALSA® MLPA® Probemix P248 MLH1-MSH2 Confirmation

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 P248 MLH1-MSH2 Confirmation product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P248 MLH1-MSH2 Confirmation**
Version	B2
Catalogue numbers	P248-025R (25 reactions) P248-050R (50 reactions) P248-100R (100 reactions)
Basic UDI-DI	872021148P2486A
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

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
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: www.mrcholland.com	
	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

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 B1, two reference probes have been replaced and three probe lengths have been adjusted.

1. Intended Purpose

The SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation 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 *MLH1* and *MSH2* genes in genomic DNA isolated from human peripheral whole blood specimens. P248 MLH1-MSH2 Confirmation is intended to confirm a potential cause for and clinical diagnosis of Lynch syndrome, and to confirm results initially detected using the SALSA MLPA Probemix P003 MLH1/MSH2. P248 MLH1-MSH2 Confirmation cannot be used to verify deletions in exon 9 of the *EPCAM* gene or the recurrent 10 Mb inversion on chromosome arm 2p detected by P003 MLH1/MSH2. It is therefore recommended to use this assay in combination with sequence analysis.

Discordant results between P248 MLH1-MSH2 Confirmation and P003 MLH1/MSH2 should be confirmed with a different technique. Most defects in the *MLH1* and *MSH2* genes are point mutations, none of which will be detected by MLPA.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

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

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types	
Test Sample	• Provided by user
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of Lynch syndrome.
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination
Positive Control Samples (Preferably)	• Provided by user

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*MLH1*/*MSH2*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	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 SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation in 47 samples from healthy individuals with normal copy number and five samples with known CNVs. The expected FRs for the corresponding copy number were found in all almost all samples tested, with a total of four ambiguous measurements having been obtained. Three of these deviations were put down to the specific sample (*MLH1* heterozygous duplication sample), as the affected probes showed variation in all performance experiments. As the remaining deviation encompassed <1% of all measurements, it was deemed negligeable.
Limit of Detection	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 SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation on four samples with known CNVs and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA, with the exception of five measurements in the prior mentioned *MLH1* heterozygous duplication sample.
Interfering substances	SNPs 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 SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. 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.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 484/525 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 509/525 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 513/525 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 521/525 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 443/525 measurements

Study	Description
	* Results are summarised for all probes across all five samples tested. Hemoglobin had the largest effect on copy number determination: final ratios within an incorrect or ambiguous range were obtained in almost all samples. Importantly, warnings or errors were obtained in all affected samples using Coffalyser.Net software. DNA extraction methods from blood remove hemoglobin and during testing of 23 samples extracted from blood the expected final ratios were found. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. EDTA, NaCl, Fe^{3+}, and heparin also had an effect on several samples. While EDTA, NaCl and Fe^{3+} led to both ambiguous measurements and false measurements being obtained, heparin only led to the production of ambiguous measurements. Ambiguous measurements would at most lead to delayed results as the assay may need to be repeated. No false positives or false negatives would ensue. In the cases where false measurements were obtained, Coffalyser.Net issued warnings, which would alert the user of an experimental issue. The IFU states that the results of the test should be interpreted by a clinical molecular geneticist or equivalent. 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.
Cross-reactivity	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.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation and found to have the expected results, with the exception of three measurements in the prior mentioned *MLH1* heterozygous duplication sample. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 2605/2625 data points, leading to a precision of 99%.
Clinical validity*	1.5-8% of LS is explained by large deletions or duplications in *MLH1* (GeneReviews) 4-16% of LS is explained by large deletions or duplications in *MSH2* (GeneReviews) *(Based on a 2011-2025 literature review)

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	Length (nt)	Probe number	Warnings
2p21	*MSH2*	Upstream	0.1 kb	196	06344-L28739	Ø
2p21	*MSH2*	Upstream	5.4 kb	355	02735-L20467	Ø »
2p21	*MSH2*	Exon 2	1.8 kb	184	07760-L09355	
2p21	*MSH2*	Exon 3	2.3 kb	178	00907-L28584	
2p21	*MSH2*	Exon 4	1.8 kb	409	07761-L07491	
2p21	*MSH2*	Exon 5	1.9 kb	150	08661-L09622	
2p21	*MSH2*	Exon 6	13.5 kb	269	20608-L28589	+
2p21	*MSH2*	Exon 7	16.0 kb	160	21740-L32470	¥
2p21	*MSH2*	Exon 8	17.4 kb	292	20610-L28275	+
2p21	*MSH2*	Exon 9	3.6 kb	391	07766-L07496	
2p21	*MSH2*	Exon 10	4.4 kb	136	20604-L28269	
2p21	*MSH2*	Exon 11	4.0 kb	456	20611-L28276	+
2p21	*MSH2*	Exon 12	1.4 kb	227	07768-L28586	
2p21	*MSH2*	Exon 13	2.0 kb	283	09140-L00579	
2p21	*MSH2*	Exon 14	2.2 kb	310	07769-L28573	
2p21	*MSH2*	Exon 15	2.1 kb	244	07770-L28570	
2p21	*MSH2*	Exon 16	38.5 kb	427	00920-L09358	
2p16.3	*KCNK12*			337	08663-L13228	¬
3p22.2	*MLH1*	Upstream (Exon 1)	0.5 kb	463	11504-L28578	Ø
3p22.2	*MLH1*	Exon 1	0.1 kb	172	01686-L28585	
3p22.2	*MLH1*	Exon 1	2.7 kb	250	07742-L28571	
3p22.2	*MLH1*	Intron 1 (Exon 2)	4.7 kb	154	07743-L09356	Ø
3p22.2	*MLH1*	Exon 3	3.4 kb	301	21741-L32471	¥
3p22.2	*MLH1*	Exon 4	2.6 kb	238	07745-L28569	
3p22.2	*MLH1*	Exon 5	1.8 kb	362	07746-L28577	
3p22.2	*MLH1*	Exon 6	3.0 kb	319	07747-L28574	
3p22.2	*MLH1*	Exon 7	0.3 kb	143	20605-L28740	+
3p22.2	*MLH1*	Intron 8 (Exon 8)	2.4 kb	481	20612-L28277	Ø
3p22.2	*MLH1*	Exon 9	3.0 kb	214	07750-L28588	+
3p22.2	*MLH1*	Exon 10	2.9 kb	346	07751-L28583	
3p22.2	*MLH1*	Exon 11	5.2 kb	220	20606-L32468	¥ +
3p22.2	*MLH1*	Exon 12	3.2 kb	400	07753-L08785	
3p22.2	*MLH1*	Exon 13	11.4 kb	208	07754-L28567	
3p22.2	*MLH1*	Exon 14	2.1 kb	256	20607-L28272	+
3p22.2	*MLH1*	Exon 15	5.2 kb	274	20609-L28274	+
3p22.2	*MLH1*	Exon 16	1.0 kb	418	01012-L00574	
3p22.2	*MLH1*	Exon 17	0.5 kb	436	01007-L00573	
3p22.2	*MLH1*	Exon 18	1.6 kb	202	08660-L28566	
3p22.2	*MLH1*	Exon 19		382	07758-L08431	
1p	Reference			166	22048-L31008	*
2p	Reference			130	19551-L26105	
3q	Reference			445	18573-L24179	
4p	Reference			232	19652-L26275	
4q	Reference			373	19096-L24983	
7p	Reference			472	19197-L25223	
9q	Reference			328	19756-L26539	
12q	Reference			190	20256-L23585	
17q	Reference			263	20636-L28350	
22q	Reference			493	22813-L32176	*

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 *MSH2* and *MLH1* 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 changes

*	New probes.
¥	Probes changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

¬	This probe is a flanking probe, 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.
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.
»	Detects the same sequence as the 148 nt probe in SALSA® MLPA® Probemix P003 MLH1/MSH2.
+	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.

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. Lynch syndrome due to *MLH1* or *MSH2* gene defects is an autosomal dominant disorder. A heterozygous deletion of one or more *MLH1* or *MSH2* exons that are present in the major transcript variants, NM_000249.4 (*MLH1*) and NM_000251.3 (*MSH2*), is expected to result in Lynch syndrome.
6. A duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. However, duplication of the complete *MLH1* or *MSH2* gene is not expected to result in disease. Please note that 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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description

Version B2-05 – 22 September 2025 (03S)
- Product description adapted to a new template.
- Intended purpose updated, specifying assay is manual and cannot be used to verify deletions in exon 9 of the *EPCAM* gene or the recurrent 10 Mb inversion on chromosome arm 2p detected by P003 MLH1/MSH2.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- SNV rs546035846 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warnings for target sequences outside of the known coding region added for probes 06344-L28739, 02735-L20467, 07743-L09356, 11504-L28578 and 20612-L28277.
- Warnings for a ligation site >20nt from the nearest exon added for probes 20608-L28589, 20610-L28275, 20611-L28276, 20605-L28740, 07750-L28588, 20606-L32468, 20607-L28272, and 20609-L28274.
- Probemix is now IVDR certified.