Instructions for Use

SALSA® MLPA® Probemix P072 MSH6-MUTYH

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD022, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P072 MSH6-MUTYH product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P072 MSH6-MUTYH**
Version	D1
Catalogue numbers	P072-025R (25 reactions) P072-050R (50 reactions) P072-100R (100 reactions)
Basic UDI-DI	872021148P0725V
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® Binning DNA SD022	SD022

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 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 C1, two mutation specific probes and eight probes for *MUTYH* have been added. Three MUTYH probes have been replaced. One MSH6 and all MLH1 probes have been removed. All reference probes have been adjusted. Several probes have a small change in length but no change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P072 MSH6-MUTYH is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *MSH6* gene and the *EPCAM-MSH2* region in order to confirm a potential cause for and clinical diagnosis of Lynch syndrome (LS), as well as deletions in the *MUTYH* gene in order to confirm a potential cause for and clinical diagnosis of *MUTYH*-associated polyposis (MAP), in genomic DNA isolated from human peripheral whole blood specimens. In addition, the presence of the two most common point mutations in the *MUTYH* gene among people from European descent, c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp), can be detected with this probemix. P072 MSH6-MUTYH is also intended for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P072 MSH6-MUTYH should be confirmed with a different technique. In particular, CNVs detected by only a single probe as well as the two *MUTYH* point mutations always require confirmation by another method. Most defects in the *MSH6* gene and the *MUTYH* gene are point mutations, which will not be detected by MLPA, with exception of the two aforementioned *MUTYH* point mutations. It is therefore recommended to use this assay in combination with sequence analysis. Of note, not all exons of *EPCAM*, *MSH2* and *MUTYH* are covered in this probemix.

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. This device can be used on fresh, frozen, or formalin-fixed paraffin embedded (FFPE) tumour tissue for RUO.

[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 normal copy number and ≤0.10 standard deviation for all probes except for mutation-specific 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 hereditary predisposition to cancer.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Binning DNA (Initial Experiment)	• SALSA Binning DNA SD022, provided by MRC Holland • Can be used in initial experiment to determine suitable bin set • Should never be used as a reference sample	
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.
Validation Samples (Required)	• In the validation experiments of this probemix, the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.	

*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 (*MSH6, EPCAM-MSH2, MUTYH*)

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.

Possible Results of MUTYH-specific Mutation Probes

Signal Strength	Mutation Status
≥10% median peak height reference probes	Mutation c.536A>G (p.Tyr179Cys) or c.1187G>A (p.Gly396Asp) is detected (expected only in positive samples)
<10% median peak height reference probes	Mutation c.536A>G (p.Tyr179Cys) or c.1187G>A (p.Gly396Asp) is not detected (expected in most samples from healthy individuals)

6. Performance Characteristics

Study	Description
Expected values for copy numbers 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 P072 MSH6-MUTYH in 47 samples from healthy individuals with normal copy number and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Expected values for point mutation detection in normal and affected populations	The mutation-specific probe will only generate a signal when the *MUTYH* c.536A>G (p.Tyr179Cys; 184 nt) and/or *MUTYH* c.1187G>A (p.Gly396Asp; 258 nt) mutation are present. Please note that background signals of the mutation-specific probe can be expected above the threshold in some cases. Users should always compare the relative peak height of the mutation-specific probe in mutation-positive samples to the relative peak height in reference samples. A clear signal (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes. The presence or absence of the mutation-specific probes was verified with SALSA MLPA Probemix P072 MSH6-MUTYH in one positive sample for the *MUTYH c.536A>G* (p.Tyr179Cys) mutation, one positive sample for the *MUTYH c.1187G>A* (p.Gly396Asp) mutation, and 47 samples from healthy individuals with normal copy numbers. The expected results were found in all tested samples.
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 P072 MSH6-MUTYH on four samples with known CNVs/mutations and on one sample without any mutation. Expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	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 SALSA MLPA Probemix P072 MSH6-MUTYH was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA of samples with known CNV/mutation status. 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 491/495 measurements Mutation: Expected signal for 30/30 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 494/495 measurements Mutation: Expected signal for 30/30 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 495/495 measurements Mutation: Expected signal for 30/30 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 494/495 measurements Mutation: Expected signal for 30/30 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 363/495 measurements Mutation: Expected signal for 30/30 measurements

* Results are summarised for all probes across all five samples tested in triplicate.

Hemoglobin had the largest effect on copy number determination: FRs within an incorrect or ambiguous range were obtained in all samples. DNA extraction methods from blood remove hemoglobin, therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. EDTA had a mild effect on copy number determination. In both cases, such values would lead to delayed results at most, as the assay may have to be repeated. No false positives or false negatives would ensue.

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, four previously genotyped samples were tested using SALSA MLPA Probemix P072 MSH6-MUTYH and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutation status over multiple days, and by multiple operators. Results showed a correct call in 2473/2475 measurements, leading to a precision of >99%.

Clinical validity*

MSH6: 0-3.5% of LS cases are caused by large deletions in *MSH6* (GeneReviews)

EPCAM-MSH2: 1-3% of LS cases are caused by large deletions in the *EPCAM-MSH2* region (Tutlewska et al. 2013)

MUTYH: <1% of MAP cases are caused by large deletions in the *MUTYH* gene (GeneReviews). Approximately 70% of patients with MAP harbours at least one of the *MUTYH* c.536A>G or *MUTYH* c.1187G>A variants (GeneReviews).

* (Based on a 2010-2025 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	Mutation	Length (nt)	Probe number	Warnings
1p34.1	*MUTYH*	Exon 16	1.2 kb		420	20900-L28954	
1p34.1	*MUTYH*	Exon 15	0.9 kb		243	15787-L29781	
1p34.1	*MUTYH*	Exon 13	0.1 kb		328	18355-L23309	
1p34.1	*MUTYH*	Exon 13	0.6 kb	c.1187G>A (G396D)	258	18417-SP0655-L23442	§ Ж
1p34.1	*MUTYH*	Exon 11	0.3 kb		202	20902-L29777	
1p34.1	*MUTYH*	Exon 9	0.2 kb		404	18420-L30955	
1p34.1	*MUTYH*	Exon 8	0.2 kb		487	20898-L29793	
1p34.1	*MUTYH*	Exon 7	0.2 kb	c.536A>G (Y179C)	184	18416-SP0654-L23441	§ Ж
1p34.1	*MUTYH*	Exon 6	0.2 kb		166	15780-L17837	
1p34.1	*MUTYH*	Exon 5	0.1 kb		232	15788-L17845	
1p34.1	*MUTYH*	Exon 4	1.1 kb		172	15781-L17838	
1p34.1	*MUTYH*	Exon 2	5.9 kb		288	15792-L29786	
1p34.1	*MUTYH*	Exon 1			135	20901-L28955	
2p21	*EPCAM*	Exon 3	11.2 kb		142	04249-L29773	
2p21	*EPCAM*	Exon 8	1.5 kb		318	20891-L29632	
2p21	*EPCAM*	Exon 9	0.1 kb		470	18132-L03603	# »
2p21	*EPCAM*	Exon 9	3.3 kb		429	13215-L29506	»
2p21	*EPCAM*	Downstream	10.3 kb		411	13146-L14626	Ø
2p21	*MSH2*	Upstream	2.6 kb		208	12006-L29778	Ø
2p21	*MSH2*	Upstream (1)	118.3 kb		358	02735-L29788	Ø »
2p16.3	*KCNK12*		261.8 kb		340	08663-L26400	¬ «
2p16.3	*MSH6*	Exon 1	0.1 kb		213	06230-L29780	+ «
2p16.3	*MSH6*	Exon 1	7.7 kb		226	21977-L31340	«
2p16.3	*MSH6*	Exon 2	0.1 kb		274	12012-L31341	
2p16.3	*MSH6*	Exon 2	5.0 kb		349	18113-L29787	
2p16.3	*MSH6*	Exon 3	2.8 kb		303	20894-L24863	
2p16.3	*MSH6*	Exon 4	0.6 kb		367	01252-L24416	
2p16.3	*MSH6*	Exon 4	0.9 kb		445	18760-L24128	
2p16.3	*MSH6*	Exon 4	3.0 kb		250	12011-L29631	
2p16.3	*MSH6*	Exon 5	0.2 kb		310	21978-L31342	
2p16.3	*MSH6*	Exon 5	1.3 kb		283	12005-L29785	
2p16.3	*MSH6*	Exon 6	0.7 kb		388	01253-L30953	
2p16.3	*MSH6*	Exon 7	0.7 kb		150	04243-L29774	
2p16.3	*MSH6*	Exon 8	0.2 kb		160	04244-L03599	
2p16.3	*MSH6*	Exon 9	0.3 kb		178	04245-L29776	
2p16.3	*MSH6*	Exon 10			196	04246-L03601	
2q13	Reference - *EDAR*				155	14199-L29775	
2q32	Reference - *SLC40A1*				190	14330-L31002	
3p14	Reference - *FLNB*				130	18835-L24359	
3q29	Reference - *OPA1*				436	06948-L29805	
4p13	Reference - *ATP8A1*				500	19675-L27455	
6p12	Reference - *PKHD1*				377	10693-L19115	
6q21	Reference - *FYN*				267	17834-L22900	
10q21	Reference - *PCDH15*				397	08792-L30954	
10q26	Reference - *UROS*				295	14829-L17169	
11q13	Reference - *SHANK2*				454	16571-L29791	
19p13	Reference - *CACNA1A*				478	13539-L29861	
22q12	Reference - *LARGE1*				220	12424-L29630	

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 *MSH6*, *EPCAM* and *MSH2* exon numbers are derived from MANE project and are based on MANE Select transcript. For *MUTYH*, exon numbers are based on the MANE Plus Clinical 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 will only generate a signal when the mutation is present. Masking of the probe signal can occur if another mutation or SNV is present **on the same allele.**
¬	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.
«	This probe is located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation.
Ж	These probes consist of three parts and have two ligation sites. A low signal of these probes can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
Ø	These probes target a sequence outside of the known coding region. Copy number alterations of only (one of) these probes are of unknown clinical significance.
#	The specificity of this probe 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.
+	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.
»	The 470 nt, 429 nt, and 358 nt probes detect the same sequences as the 481 nt, 472 nt and 148 nt probes in SALSA® MLPA® Probemix P003 MLH1/MSH2.

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, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.
6. 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, especially in solid tumours with more chaotic karyotypes.
7. MAP is an autosomal recessive disorder, thus, only biallelic mutations in the *MUTYH* gene are considered clinically relevant for a MAP diagnosis.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. A simultaneous deletion of the 470 and 429 nt exon 9 EPCAM probes is a strong indication that the 3' end of *EPCAM* is disrupted, which can lead to promoter hypermethylation and subsequently inactivation of *MSH2* in EpCAM expressing tissues (Ligtenberg et al. 2009). Therefore, if a somatic/tumour sample is available, we recommend using SALSA® MLPA® Probemix ME011 Mismatch Repair Genes to test the methylation status of the *MSH2* promoter.
2. The clinical significance of a deletion or duplication of only one of the probes targeting the *EPCAM-MSH2* region is not yet clear/clearly established.
3. The two MUTYH mutation-specific probes are only intended to determine the presence (or absence) of the mutation, and are not meant to determine the zygosity of the mutation. Follow-up with another method is advised.
4. SALSA MLPA Probemix P072 should not be used to confirm results of SALSA® MLPA® P003 MLH1/MSH2, and vice versa, as some probes (») share the same ligation site. In case CNVs are found in the *EPCAM-MSH2* region, a different method should be used to confirm the results.
5. Exon 3, 10, 12 and 14 of *MUTYH* are not covered in this probemix. For additional coverage, SALSA® MLPA® Probemix P378 MUTYH is available.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Ligtenberg MJ et al. (2009). Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. *Nat Genet.* 41:112-117.
2. Tutlewska K et al. (2013). Germline deletions in the EPCAM gene as a cause of Lynch syndrome – literature review. *Hereditary Cancer in Clinical Practice.* 11:9.

Implemented changes in the product description
Version D1-06 – 29 September 2025 (03S) - Intended purpose updated, specifying that the assay is manual. CNV type detected in *MSH6*, *MUTYH* and *EPCAM-MSH2* region limited to deletions. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - SNV rs200872702 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. - Warning for target sequences outside of the known coding region added for the 13146-L14626, 12006-L29778 and 02735-L29788 probes. - Warning for probes with a ligation site >20 nt away from the nearest exon added for the 06230-L29780 probe. - Warning for probes targeting the same sequences as those present in P003 MLH1/MSH2 added for probes 18132-L03603, 13215-L29506 and 02735-L29788. - A probemix-specific precaution was added to specify the inheritance pattern of MAP and the clinical relevance of obtained results with SALSA MLPA Probemix P072 MSH6-MUTYH. - Limitation regarding follow-up with SALSA MLPA Probemix ME011 Mismatch Repair Genes updated for clarification. - Limitation added regarding the clinical significance of a deletion in only one of the probes targeting the *EPCAM-MSH2* region. - Limitation added to clarify the purpose of the MUTYH mutation-specific probes. - Limitation regarding the use of P003 MLH1/MSH2 as a confirmation mix was added. - Limitation regarding the coverage of *MUTYH* in this probemix added. - Probemix is now IVDR certified.