Product Description
SALSA® MLPA® Probemix P499-A1 EPCAM

To be used with the MLPA General Protocol.

Version A1
First release.

Catalogue numbers
- **P499-025R:** SALSA® MLPA® Probemix P499 EPCAM, 25 reactions
- **P499-050R:** SALSA® MLPA® Probemix P499 EPCAM, 50 reactions
- **P499-100R:** SALSA® MLPA® Probemix P499 EPCAM, 100 reactions

SALSA® MLPA® Probemix P499 EPCAM (hereafter: P499 EPCAM) is to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

Volumes and ingredients

Volumes			Ingredients
P499-025R	**P499-050R**	**P499-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemix 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).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, 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.

Certificate of Analysis
Information regarding quality tests and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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.

General information
SALSA® MLPA® Probemix P499 EPCAM is a **research use only (RUO)** assay for the detection of deletions or duplications in the region containing the *EPCAM* gene and the first two exons of the *MSH2* gene, which is associated with Lynch syndrome (end of *EPCAM* and *MSH2*) and congenital tufting enteropathy (*EPCAM*).

Lynch syndrome, formerly known as hereditary non-polyposis colorectal cancer (HNPCC), is an adult-onset hereditary cancer susceptibility syndrome predisposing to several cancer types, the most prevalent being

colorectal cancer, endometrial cancer, ovarian cancer, gastric cancer and small bowel cancer. It is a dominantly inherited syndrome caused by heterozygous germline mutations in one of the four major DNA mismatch repair genes: *MLH1*, *MSH2*, *MSH6* or *PMS2*. Another cause of Lynch syndrome is a deletion of the 3' part of *EPCAM*, leading to constitutional epigenetic silencing of the downstream *MSH2* gene (Lynch et al. 2015). Disruption of the last exon of *EPCAM* (exon 9) can lead to methylation of the adjacent *MSH2* promoter and inactivation of *MSH2* (Ligtenberg et al. 2009, Kovacs et al. 2009). The estimated contribution to Lynch syndrome is 20-40% for *MSH2* and <10% for *EPCAM*.
Congenital tufting enteropathy (CTE) is an autosomal recessive disorder caused by pathogenic variants in *EPCAM*. The estimated incidence is 1 in 50,000-100,000 live births. Symptoms are intractable diarrhoea, malnutrition, and growth failure in early infancy (Zhao et al. 2026).

The *EPCAM* gene (9 exons) spans ~18 kb of genomic DNA. The intergenic region between *EPCAM* and *MSH2* spans ~16 kb. The *MSH2* gene (16 exons) spans ~80 kb of genomic DNA. Both genes are located on 2p21, ~47 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1211/ and https://omim.org/entry/613217.

This product is not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide
Matched Annotation from NCBI and EMBL-EBI (MANE): https://www.ncbi.nlm.nih.gov/refseq/MANE
Tark – Transcript Archive: https://tark.ensembl.org

Exon numbering
The *EPCAM* and *MSH2* exon numbering used in this P499-A1 EPCAM product description are the exon numbering derived from MANE project (release version 1.5) based on MANE Select transcripts NM_002354.3 and NM_000251.3, respectively. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
P499-A1 EPCAM contains 32 MLPA probes with amplification products between 130 and 398 nucleotides (nt). This includes 11 probes for the *EPCAM* gene, seven probes for the intergenic region (between *EPCAM* and *MSH2*) and four probes for the *MSH2* gene. In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.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.mrcholland.com.

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

MLPA technique
The principles of MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of different reference samples from unrelated individuals should be included in each MLPA experiment for data normalisation. Reference samples should be derived from individuals who are from families without a history of lynch syndrome or congenital tufting enteropathy. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

Data analysis
Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net is freely downloadable at www.mrcholland.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 standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication/gain	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	1.75 < FR < 2.15
Ambiguous copy number	All other values

Please note that Coffalyser.Net also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. single nucleotide variants (SNVs), point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also 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: https://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 (as described for *DMD* by 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.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *EPCAM* and *MSH2* genes are small (point) mutations, none of which will be detected by using P499 EPCAM.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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.

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 in sequence data indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by 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.

***EPCAM* and *MSH2* mutation database**
https://databases.lovd.nl/shared/genes/EPCAM and https://databases.lovd.nl/shared/genes/MSH2. We strongly encourage users to deposit positive results in the Leiden Open Variation Database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on https://varnomen.hgvs.org.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *EPCAM* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

Table 1. P499-A1 EPCAM

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*EPCAM*	Intergenic region	*MSH2*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 18946-L27359	5q			
138	**EPCAM probe** 04249-L24406		**Exon 3**		
148	**MSH2 probe** 12036-L02162			*MSH2* upstream	
154	**EPCAM probe** 23541-L14403			*EPCAM* downstream	
160	**MSH2 probe** 00906-L00494				**Exon 2**
170	**EPCAM probe** 23536-L26396		**Exon 2**		
178	Reference probe 18842-L30565	3p			
184	**MSH2 probe** 06227-L07711			*MSH2* upstream	
190	Reference probe 12780-L28100	2q			
196	**EPCAM probe** 19592-L26612		**Exon 1**		
203	**EPCAM probe** 23540-L33343		**Exon 8**		
208	Reference probe 10644-L30437	9q			
214	**MSH2 probe** 13145-L14402			*MSH2* upstream	
220	**EPCAM probe** 22424-L31926		**Exon 9**		
227	**EPCAM probe** 23537-L26192		**Exon 4**		
232	Reference probe 05709-L30439	3q			
238	Reference probe 08070-L07851	9p			
247	**MSH2 probe** 06226-L07709				**Exon 1**
252	**EPCAM probe** 19731-L26773		**Exon 1**		
263	Reference probe 15809-L30975	19q			
271	**EPCAM probe** 23539-L26194		**Exon 7**		
280 Ø	**MSH2 probe** 23533-L33344				Intron 1
290	**EPCAM probe** 19594-L26615		**Exon 5**		
306	**MSH2 probe** 23534-L26614			*MSH2* upstream	
314	**EPCAM probe** 13131-L33342		**Exon 9**		
323	Reference probe 09065-L30441	19p			
331	**EPCAM probe** 23542-L26616			*EPCAM* downstream	
342	**EPCAM probe** 23538-L26774		**Exon 6**		
350	**MSH2 probe** 23535-L29778			*MSH2* upstream	
366	**MSH2 probe** 16175-L33370				**Exon 1**
386	Reference probe 13404-L17499	6q			
398	Reference probe 00973-L30443	10q			

[a] See section Exon numbering on page 2 for more information.

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. *EPCAM/MSH2* probes arranged according to chromosomal location

Length (nt)	MLPA probe	Gene exon[a]	Ligation site[b]	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
			***EPCAM* gene**		
			NM_002354.3		
252	19731-L26773	Exon 1	73 nt before exon 1	GCGGCCAGAGGT-GAGCAGTCCCGG	0.3 kb
		start codon	*196-198 (Exon 1)*		
196	19592-L26612	Exon 1	233-234	CGGGCTTCTGCT-TGCCGCGGCGAC	4.0 kb
170	23536-L26396	Exon 2	316-317	ACTGCTTTGTGA-ATAATAATCGTC	0.5 kb
138	04249-L24406	Exon 3	571-572	CTGGGGTCAGAA-GAACAGACAAGG	1.2 kb
227	23537-L26192	Exon 4	625-626	TTTATAGCTGGA-TCATCATTGAAC	1.8 kb
290	19594-L26615	Exon 5	746-747	TATCACGAGTAT-TTTGGTATGATT	2.0 kb
342	23538-L26774	Exon 6	846-847	TATTATTTTGAA-AAAGATGTGAGT	0.7 kb
271	23539-L26194	Exon 7	868-869	AAGGTGAATCCT-TGTTTCATTCTA	5.4 kb
203	23540-L33343	Exon 8	1065-1066	GTTATTTCCAGA-AAGAAGAGAATG	1.5 kb
		stop codon	*1138-1140 (Exon 9)*		
314	13131-L33342	Exon 9	1189-1190	AAATGGACACAA-ATTACAAATGTG	0.1 kb
220	22424-L31926	Exon 9	1320-1319 *reverse*	GGTCAAATTTCA-AGATTGGTAAAG	3.3 kb
			Intergenic region		
154	23541-L14403	Downstream	3.1 kb after *EPCAM* exon 9	TCATTCCCAGAA-TAACTAAAATCT	5.6 kb
331	23542-L26616	Downstream	8.7 kb after *EPCAM* exon 9	TACTGGATTCTA-TCAATCATGCTA	4.2 kb
306	23534-L26614	Upstream	3.2 kb before *MSH2* exon 1	TTCTCAGTATAC-AGTCTTGCTGCA	0.4 kb
350	23535-L29778	Upstream	2.8 kb before *MSH2* exon 1	AGAGATTTCTAA-ATTGAGCTGATT	2.4 kb
214	13145-L14402	Upstream	0.3 kb before *MSH2* exon 1	CCGGGCACATTA-CGAGCTCAGTGC	0.1 kb
184	06227-L07711	Upstream	0.2 kb before *MSH2* exon 1 *reverse*	GACGCGCATCCT-TAGTAGAGCTCC	0.1 kb
148	12036-L02162	Upstream	156 nt before *MSH2* exon 1	GCGTGCGCGGGA-AGCTGGGCCGCG	0.2 kb
			***MSH2* gene**		
			NM_000251.3		
247	06226-L07709	Exon 1	5-6	GGGTCGCGCATT-TTCTTCAACCAG	0.2 kb
		start codon	*37-39 (Exon 1)*		
366	16175-L33370	Exon 1	163-164	GGGGCGACTTCT-ATACGGCGCACG	0.3 kb
280	23533-L33344	Intron 1	0.3 kb after exon 1 *reverse*	GAACTAGAACAA-TGCATTAAAATG	4.8 kb
160	00906-L00494	Exon 2	339-340	TATAGAGTTGAA-GTTTATAAGAAT	
		stop codon	*2839-2841 (Exon 16)*		

[a] See section Exon numbering on page 2 for more information.
[b] Ligation sites are relative to the start of the NM_ sequence, and not relative to the coding sequence.
[c] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

SNVs located in the target sequence of a probe can influence probe hybridisation and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

Related products
For related products, see the product page on our website.

References

- Kovacs ME et al. (2009). Deletions removing the last exon of *TACSTD1* constitute a distinct class of mutations predisposing to Lynch syndrome. *Hum Mutat.* 30:197-203.
- 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.
- Lynch HT et al. (2015). Milestones of Lynch syndrome: 1895-2015. *Nat Rev Cancer.* 15:181-194.
- 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.
- Zhao L et al. (2026). Genotype-phenotype correlations in *EPCAM*-associated congenital tufting enteropathy: a case report and systematic review. *Front Pediatr*. 14:1830464.

P499 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 – 28 September 2026 (05P) - Not applicable, new document.

More information: www.mrcholland.com; www.mrcholland.eu	
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