Product Description SALSA® MLPA® Probemix P003-D1 MLH1/MSH2

To be used with the MLPA General Protocol.

Version D1. As compared to version C1, two probes have been included that detect a recurrent 10 Mb inversion disrupting *MSH2*. In addition, one reference probe has been replaced (Table 1). For complete product history see page 12.

Catalogue numbers:

- **P003-025R:** SALSA MLPA probemix P003 MLH1/MSH2, 25 reactions.
- **P003-050R:** SALSA MLPA probemix P003 MLH1/MSH2, 50 reactions.
- **P003-100R:** SALSA MLPA probemix P003 MLH1/MSH2, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mlpa.com.

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

Intended use: This SALSA MLPA probemix P003 MLH1/MSH2 is an in vitro diagnostic (IVD)[1] or research use only (RUO) assay for the detection of exon deletion(s) or duplication(s) in specific regions of the *MLH1*, *MSH2* and *EPCAM* genes, as well as a recurrent 10 Mb inversion on chromosome arm 2p which disrupts the *MSH2* gene, in order to confirm a potential cause and clinical diagnosis for Lynch syndrome. This product can also be used for molecular genetic testing of at-risk family members.

This assay is for use with peripheral blood derived genomic DNA and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P003 MLH1/MSH2 probemix should be verified by using the SALSA MLPA probemix P248 MLH1/MSH2 Confirmation or a different technique. This verification must always be performed for copy number changes detected by only a single probe. P248 MLH1/MSH2 Confirmation cannot be used to verify deletions or duplications in *EPCAM*.

This assay is not intended to be used as a standalone assay for clinical decisions. Most defects in the *MLH1* and *MSH2* genes are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Germline defects in the *MLH1* and *MSH2* genes are the most frequent cause of a hereditary predisposition to Lynch syndrome (formerly known as hereditary non-polyposis colorectal cancer; HNPCC). Lynch syndrome is an inherited cancer of the digestive tract, particularly the colon and rectum. In addition, defects in the *MLH1* and *MSH2* genes increase the risk for numerous other cancers due to impaired DNA repair. More information is available on http://www.ncbi.nlm.nih.gov/books/NBK1211/.

Approximately 50% of Lynch syndrome cases are attributed to mutations in *MLH1*, 40% are attributed to mutations in *MSH2*, and 1-3% are due to *EPCAM* deletions. In addition, 10-20% of the cases can be explained by mutations in the *MSH6* and *PMS2* genes. Among the various defects in the *MLH1* and *MSH2* genes that have been found in patients are deletions and duplications of complete exons, which are usually

missed by standard sequence analysis. The MLPA technique can detect most of these deletions and duplications and therefore complements sequence analysis of the *MLH1* and *MSH2* genes.

Gene structure: The *MLH1* gene spans 57 kb on chromosome 3p22.2, and *MSH2* spans 80 kb on chromosome 2p21. The *MLH1* LRG_216 is identical to GenBank NG_007109.2 and the *MSH2* LRG_218 is identical to GenBank NG_007110.2. Both are available at www.lrg-sequence.org/.

Transcript variants:
MLH1 - Multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/4292. *MLH1* transcript variant 1 (NM_000249.3, 2662 nt, coding sequence 199-2469) represents the most abundant transcript, encoding the full-length protein. This sequence is a reference standard in the NCBI RefSeqGene project. In Table 2a, the ligation sites of the *MLH1* MLPA probes are indicated according to this sequence which contains 19 exons. The ATG translation start site is located in exon 1 and the stop codon is located in exon 19.
MSH2 - Multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/4436. *MSH2* transcript variant 1 (NM_000251.2, 3226 nt, coding sequence 126-2930) represents the longer transcript, encoding the longer isoform. This sequence is a reference standard in the NCBI RefSeqGene project. The ligation sites of the *MSH2* MLPA probes are indicated according to this sequence which contains 16 exons. The ATG translation start site is located in exon 1 and the stop codon is located in exon 16. The other transcript variant, NM_001258281.1, lacks an alternate segment of the first exon, including the translation start site, and is shorter at the N-terminus.

Exon numbering: The exon numbering used in this P003-D1 MLH1/MSH2 product description is the exon numbering for *MLH1* from the RefSeq transcript NM_000249.3, which is identical to the LRG_216 sequence, and for *MSH2* from the RefSeq transcript NM_000251.2, which is identical to the LRG_218 sequence. The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

Probemix content: This SALSA MLPA probemix P003 MLH1/MSH2 version D1 contains 50 MLPA probes with amplification products between 130 and 499 nt (Table 1) including 39 probes for the *MLH1/MSH2/EPCAM* gene regions (Table 2), 2 probes that only generate signals on samples containing the recurrent 10 Mb inversion that disrupts *MSH2* (Wagner et al. 2002, Chen 2008, Rhees et al. 2014), and 9 reference probes that detect sequences outside this region. The identity of the genes detected by the reference probes is available online (www.mlpa.com).

One probe is present for each of the 19 exons of the *MLH1* gene and for each of the 16 exons of the *MSH2* gene. Two probes are included for exon 9 of *EPCAM* (formerly known as *TACSTD1*), a gene located just upstream of *MSH2*. Deletions of this last exon of *EPCAM* can result in silencing of the *MSH2* gene.

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.mlpa.com.

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

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

MLPA technique validation: Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing 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 from human peripheral blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference 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. Reference samples should be derived from unrelated individuals who are from families without a history of hereditary predisposition to cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: The NIBSC Institute in the U.K. provides a kit with 5 DNA samples containing *MLH1* or *MSH2* exon deletions or amplifications: http://www.nibsc.org/documents/ifu/11-218-xxx.pdf.

SALSA Binning DNA SD052: The SD052 Binning DNA provided with this probemix can be used as Binning DNA sample for binning of two inversion specific probes (265 nt probe 20091-SP0917-L28216 and 317 nt probe 20090-SP0916-L28222 for a 10 Mb inversion with breakpoint in intron 7 of *MSH2*). SD052 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequences detected by the above mentioned probes. Inclusion of one reaction with 5 µl SD052 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signals, as for this purpose true inversion positive patient samples or cell lines should be used. It is strongly advised to use DNA sample and reference DNA samples extracted with the same method and derived from the same source of tissue. For further details, please consult the SD052 Binning DNA product description provided.

Performance characteristics: Large deletions account for ~20% of variants in *MSH2*, ~5% of variants in *MLH1* and for all *EPCAM* variants that cause Lynch syndrome (http://www.ncbi.nlm.nih.gov/books/NBK1211/), and the great majority of these can be detected by this P003 MLPA probemix. Furthermore, the recurrent 10 Mb inversion of chromosome 2 which has one breakpoint in intron 7 of the *MSH2* gene can also be detected by this probemix (Wagner et al. 2002, Chen 2008, Rhees et al. 2014). *MLH1* or *MSH2* duplications have been rarely reported, but should also be detectable with this probemix. Note that the P003 MLH1/MSH2 probemix does not contain probes for the detection of *MSH6* and *PMS2* genes, which are also implicated in Lynch syndrome. The analytical sensitivity and specificity for the detection of deletions in the aforementioned genes using point mutation negative samples with Lynch syndrome is very high and can be considered >99%.

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.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 expected results for MLH1/MSH2 region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication) and occasionally 4 (heterozygous triplication). Homozygous deletions may occur, but are extremely rare.

The standard deviation of all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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: http://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 (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. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Notes P003 MLH1-MSH2 results**:**

- Lynch syndrome due to *MLH1/MSH2/EPCAM* gene defects is an autosomal dominant disorder. Inactivation of a single copy of the *MLH1/MSH2* gene or deletion of the last *EPCAM* exon is expected to be pathogenic.
- A heterozygous deletion of one or more *MLH1* or *MSH2* exons that are present in the major transcript variants, NM_000249.3 (*MLH1*) and NM_000251.2 (*MSH2*), is expected to result in Lynch syndrome.
- Simultaneous deletion of the 472 and 481 nt EPCAM probes is a strong indication that the last *EPCAM* exon (exon 9) is disrupted, which can lead to methylation and inactivation of *MSH2* (Ligtenberg et al. 2009, Kovacs et al. 2009).
- The presence of peaks at 265 and 317 nt, or only at 265 nt, is an indication that the 10 Mb chromosome 2 inversion is present in the sample DNA (Wagner et al. 2002, Chen 2008, Rhees et al. 2014). These probes do not generate a signal on normal samples. Please note that the peak height of these two probes can be strongly affected by DNA fragmentation. The 20091-SP0917-L28216 probe present at 265 nt and the 20090-SP0916-L28222 probe present at 317 nt require an intact DNA fragment to bind to of at least 220 nt or 520 nt, respectively, in inversion positive DNA samples, which is much longer than the length requirements for other MLPA probes (60-80 nt). DNA samples in which both probes show a clear peak, as well as samples in which only a clear peak for one of the two probes is observed, should be further investigated even when these peaks are much lower as obtained on the SD052 Binning DNA

sample. A non-specific peak close to the 265 nt peak can appear if the ligation reaction is performed at a temperature below 54°C.
- 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 the remark above on duplications that include the first or last exon of a gene.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *MLH1* and *MSH2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P003 MLH1/MSH2.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and does not detect translocations or copy number neutral inversions, except the 10 Mb inversion with breakpoint in intron 7 of *MSH2*. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in the genes or chromosomal regions *do* exist but remain undetected.
- Sequence changes (e.g. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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: Deletions or duplications obtained with the P003 MLH1/MSH2 probemix must be verified by using SALSA MLPA probemix P248 MLH1/MSH2 Confirmation or a different 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. Almost all probes included in probemix P248 MLH1/MSH2 Confirmation have different ligation sites from those in P003, except for the 148 nt exon 1 *MSH2* probe. The P072 MSH6 MLPA probemix contains additional probes for *EPCAM* and the region between *MSH2* and *EPCAM*. In addition, the P072 probemix can be used to detect copy number variations in the *MSH6* gene.

Copy number changes detected by only a single probe always require confirmation by the probemix P248 MLH1/MSH2 Confirmation or 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 indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

MLH1/MSH2 mutation database: http://www.insight-group.org/; http://www.lovd.nl. We encourage users to deposit positive results in the LOVD database of the International Society for Gastrointestinal Hereditary Tumours (INSiGHT). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *MLH1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P003-D1 MLH1/MSH2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] reference	*MLH1*	*MSH2 / EPCAM*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L21056	5q31		
136	Reference probe 00981-L00566	10p11		
142	**MLH1 probe** 14701-L19726		**Exon 1**	
148 »	**MSH2 probe** 12036-L02162			**Exon 1**
154	**MLH1 probe** 01008-L00577		**Exon 2**	
160	**MSH2 probe** 00906-L00494			**Exon 2**
166	**MLH1 probe** 00888-L00476		**Exon 3**	
172	**MSH2 probe** 01029-L00601			**Exon 3**
178	**MLH1 probe** 00889-L23928		**Exon 4**	
184	**MSH2 probe** 00908-L00496			**Exon 4**
190	**MSH2 probe** 11287-L12006			**Exon 1**
196	Reference probe 17056-L20134	7q31		
203 ¥	**MLH1 probe** 20360-L28308		**Exon 5**	
211	**MSH2 probe** 00909-L00497			**Exon 5**
217	**MSH2 probe** 13145-L14624			**Exon 1**
224 ¥	**MLH1 probe** 14702-L28215		**Exon 6**	
231	**MSH2 probe** 15298-L17064			**Exon 6**
236	Reference probe 15941-L18067	2q24		
242	**MLH1 probe** 00892-L16355		**Exon 7**	
249 «	**MSH2 probe** 11634-L16356			**Exon 7**
256	**MLH1 probe** 00893-L00481		**Exon 8**	
265 * §	**Inversion probe** 20091-SP0917-L28216			**Intron 7**
269 ¥	**MSH2 probe** 00912-L28217			**Exon 8**
278 ¥ +	**MLH1 probe** 00894-L28218		**Exon 9**	
287 *	Reference probe 18920-L25191	1p36		
292 ¥	**MSH2 probe** 00913-L28221			**Exon 9**
301	**MLH1 probe** 00895-L00483		**Exon 10**	
310	**MSH2 probe** 11288-L12007			**Exon 10**
317 * §	**Inversion probe** 20090-SP0916-L28222			**Intron 7**
326 ¥	**MLH1 probe** 00896-L18364		**Exon 11**	
332 ¥	**MSH2 probe** 00915-L28223			**Exon 11**
340 ¥ ∫	**MLH1 probe** 14703-L28224		**Exon 12**	
346	**MSH2 probe** 18133-L23925			**Exon 12**
355	**MLH1 probe** 00898-L23926		**Exon 13**	
364	**MSH2 probe** 01013-L00575			**Exon 13**
375	Reference probe 00681-L11147	4q25		
382	**MLH1 probe** 00899-L00586		**Exon 14**	
391	**MSH2 probe** 00918-L00506			**Exon 14**
401	**MLH1 probe** 00900-L00488		**Exon 15**	
409	**MSH2 probe** 00919-L00585			**Exon 15**
418	**MLH1 probe** 01009-L00576		**Exon 16**	
427	**MSH2 probe** 01053-L14623			**Exon 16**
436	**MLH1 probe** 01030-L00602		**Exon 17**	
445	**MLH1 probe** 01031-L00603		**Exon 18**	
454	**MLH1 probe** 12094-L12994		**Exon 19**	
463	Reference probe 00979-L00568	10p14		
472	**EPCAM probe** 13147-L14404			**(Exon 9)**
481	**EPCAM probe** 18132-L24050			**(Exon 9)**
490 «	Reference probe 04274-L24051	13q12		
499	Reference probe 14882-L21050	14q11		

(a) The exon numbering used in this P003-D1 MLH1/MSH2 product description is the exon numbering for *MLH1* from the RefSeq transcript NM_000249.3, which is identical to the LRG_216 sequence, and for *MSH2* from the RefSeq transcript NM_000251.2, which is identical to the LRG_218 sequence. The exon numbering

and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

* New in version D1 (from lot D1-0815 onwards).
¥ Changed in version D1 (from lot D1-0815 onwards). Small change in length, no change in sequence detected.
« Probe located within, or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
+ See note on the 278 nt *MLH1* exon 9 probe below Table 2a.
ʃ See note on the 340 nt *MLH1* exon 12 probe below Table 2a.
§ Mutation-specific probe. This probe will only generate a signal when the 10 Mb inversion with breakpoint in intron 7 of *MSH2* is present. This probe consists of three parts and has two ligation sites. See note about this probe on page 4, section interpretation of results.
» Detects the same sequence as the 355 nt probe (02735-L20467) in SALSA MLPA probemix P248 MLH1/MSH2 Confirmation.

Table 2. P003 probes arranged according to chromosomal location

Table 2a. *MLH1* gene

Length (nt)	SALSA MLPA probe	*MLH1* Exon[a]	Ligation site[b] NM_000249.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*199-201 (exon 1)*		
142	14701-L19726	Exon 1	205-206	CCAAAATGTCGT-TCGTGGCAGGGG	3.1 kb
154	01008-L00577	Exon 2	383-384	GATTCAGATCCA-AGACAATGGCAC	4.3 kb
166	00888-L00476	Exon 3	463-464	TGCAGTCCTTTG-AGGATTTAGCCA	3.4 kb
178	00889-L23928	Exon 4	527-528	CATAAGCCATGT-GGCTCATGTTAC	2.6 kb
203	20360-L28308	Exon 5	627-628	AAACCATGTGCT-GGCAATCAAGGG	1.8 kb
224	14702-L28215	Exon 6	664-665	TGGAGGACCTTT-TTTACAACATAG	3.0 kb
242	00892-L16355	Exon 7	764-765	ACACAATGCAGG-CATTAGTTTCTC	0.2 kb
256	00893-L00481	Exon 8	818-819	TGTTAGGACACT-ACCCAATGCCTC	2.4 kb
278 +	00894-L28218	Exon 9	920-921	CCTAGCCTTCAA-AATGAATGGTTA	3.1 kb
301	00895-L00483	Exon 10	1019-1020	TTCCTTGAGAAA-AGCCATAGAAAC	2.9 kb
326	00896-L18364	Exon 11	1190-1191	GCAGCACATCGA-GAGCAAGCTCCT	5.5 kb
340 ʃ	14703-L28224	Exon 12	1482-1483	AGGCAGCAAGAT-GAGGAGATGCTT	3.0 kb
355	00898-L23926	Exon 13	1665-1666	TCCCGAAAGGAA-ATGACTGCAGCT	11.4 kb
382	00899-L00586	Exon 14	1799-1800	CGTGGGCTGTGT-GAATCCTCAGTG	2.1 kb
401	00900-L00488	Exon 15	1892-1893	CCAGATACTCAT-TTATGATTTTGC	5.3 kb
418	01009-L00576	Exon 16	1967-1968	CATGCTTGCCTT-AGATAGTCCAGA	1.0 kb
436	01030-L00602	Exon 17	2172-2173	ATCTTCATTCTT-CGACTAGCCACT	0.4 kb
445	01031-L00603	Exon 18	2269-2268 reverse	CTCCTCAGATAT-GTACTGCTTCCG	1.8 kb
454	12094-L12994	Exon 19	2595-2594 reverse	TATCAGAAGGCA-AGTATAAGTCTT	
		stop codon	*2467-2469 (exon 19)*		

(a) The exon numbering used in this P003-D1 MLH1/MSH2 product description is the exon numbering for *MLH1* from the RefSeq transcript NM_000249.3, which is identical to the LRG_216 sequence. The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

(b) Ligation sites of the P003 MLH1 MLPA probes are indicated according to RefSeq sequence NM_000249.3 containing 19 exons.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

+ The MLH1 exon 9 probe in the previous version could give a duplication signal that could not be reproduced by the MLH1 exon 9 probe in the P248 MLH1/MSH2 Confirmation probemix. This apparent duplication was probably caused by the c.702G>A variant (rs35908749), located 20 nt from the ligation site. In the current D1 version (lot 0815 onwards) this probe has been adjusted, resulting in normal results when a heterozygous positive sample for this SNP is analysed. Please notify us if you still obtain different results with this probe than with the corresponding probe in the P248 probemix.

∫ The 340 nt MLH1 exon 12 probe has been found to give false positive deletion/duplication results. This is probably due to an unusual low sensitivity of this probe to sample DNA depurination. Depurination of sample DNA can occur in FFPE derived samples or in samples with insufficient buffering capacity. The MLH1 exon 12 probe in the P248 probemix does not have this problem and can be used for confirmation. More information on sample DNA depurination is available on www.mlpa.com.

Table 2b. *MSH2* gene

Length (nt)	SALSA MLPA probe	*MSH2* Exon[(a)]	Ligation[(b)] site	Partial sequence[(c)] (24 nt adjacent to ligation site)	Distance to next probe
			***EPCAM* gene**		
			NM_002354.2		
	EPCAM stop codon		*1301-1303 (exon 9)*		
481 #	18132-L24050	*EPCAM* Exon 9	1352-1353	AAATGGACACAA-ATTACAAATGTG	0.1 kb
472	13147-L14404	*EPCAM* Exon 9	1483-1482 reverse	GGTCAAATTTCA-AGATTGGTAAAG	16.0 kb to MSH2
			***MSH2* gene**		
			NM_000251.2		
		start codon	*126-128 (exon 1)*		
217	13145-L14624	Exon 1	257 nt before exon 1	CCGGGCACATTA-CGAGCTCAGTGC	0.2 kb
148 »	12036-L02162	Exon 1	67 nt before exon 1	GCGTGCGCGGGA-AGCTGGGCCGCG	0.7 kb
190	11287-L12006	Exon 1	269 nt after exon 1 reverse	GAACTAGAACAA-TGCATTAAAATG	4.8 kb
160	00906-L00494	Exon 2	428-429	TATAGAGTTGAA-GTTTATAAGAAT	1.7 kb
172	01029-L00601	Exon 3	576-577	TTGTGGGTGTTA-AAATGTCCGCAG	2.3 kb
184	00908-L00496	Exon 4	863-864	CGGTTGTTGAAA-GGCAAAAAGGGA	1.8 kb
211	00909-L00497	Exon 5	943-944	ACTGTCTGCGGT-AATCAAGTTTTT	2.1 kb
231	15298-L17064	Exon 6	1118-1119	GCCTTGCTGAAT-AAGTGTAAAACC	13.5 kb
249 «	11634-L16356	Exon 7	1322-1323	AGACAAGCAGCA-AACTTACAAGAT	15.7 kb
269	00912-L28217	Exon 8	1449-1450	CTCCTCTTACTG-ATCTTCGTTCTG	17.5 kb
292	00913-L28221	Exon 9	1536-1537	AATTCCTTGTAA-AACCTTCATTTG	3.9 kb
310	11288-L12007	Exon 10	189 nt after exon 10	GACTGAAGTGGT-ACTTTGGGTCTA	4.0 kb
332	00915-L28223	Exon 11	1832-1831 reverse	GCTTCTTCATAT-TCTGTTTTATTT	4.1 kb
346	18133-L23925	Exon 12	1980-1981	CACCTGTTCCAT-ATGTACGACCAG	1.3 kb
364	01013-L00575	Exon 13	2196-2197	TCATGGCCCAAA-TTGGGTGTTTTG	1.9 kb
391	00918-L00506	Exon 14	2403-2404	CCTACGATGGAT-TTGGGTTAGCAT	2.5 kb
409	00919-L00585	Exon 15	2686-2687	ACTTGAGGAGTT-TCAGTATATTGG	2.0 kb
427	01053-L14623	Exon 16	2762-2763	GTGTTTCAGCAA-GGTGAAAAAATT	
		stop codon	*2928-2930 (exon 16)*		
265 §	20091-0917-L28216	Intron 7	This probe will only generate a signal on mutation positive DNA samples (10 Mb inversion with breakpoint in *MSH2* intron 7		
317 §	20090-0916-L28222	Intron 7	This probe will only generate a signal on mutation positive DNA samples (10 Mb inversion with breakpoint in *MSH2* intron 7		

(a) The exon numbering used in this P003-D1 MLH1/MSH2 product description is the exon numbering for *MSH2* from the RefSeq transcript NM_000251.2, which is identical to the LRG_218 sequence. The exon numbering and NM sequence used is from 07/2018, but can be changed by NCBI after the release of the product description.

(b) Ligation sites of the P003 MSH2 MLPA probes are indicated according to RefSeq sequence NM_000251.2 containing 16 exons.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« Probe located within, or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

§ Mutation-specific probe. This probe will only generate a signal when the 10 Mb inversion with breakpoint in intron 7 of *MSH2* is present. This probe consists of three parts and has two ligation sites. See note about this probe on page 4, section interpretation of results.
» Detects the same sequence as the 355 nt probe (02735-L20467) in SALSA MLPA probemix P248 MLH1/MSH2 Confirmation.
This probe's specificity 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.

Related SALSA MLPA probemixes

	Condition		Gene	Probemix
COLORECTAL CANCER (CRC) PROBEMIXES	Lynch Syndrome (HNPCC)		*MLH1*	P003, ME011, P248 (Confirmation of P003), ME042
			MSH2	P003, ME011, P248 (Confirmation of P003)
			MSH6	P072, ME011
			PMS2	P008, ME011
			EPCAM	P003, P072
	Polyposis Syndrome	MAP	*MUTYH*	P378, P043, P072
		AFAP	*APC*	P043
		FAP	*APC*	P043

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P003 Product history	
Version	*Modification*
D1	Two probes specific for the recurrent 10 Mb inversion on chr. 2p have been added; and one reference probe has been replaced.
C1	Two reference probes have been replaced and two extra reference probes have been added.
B2	The 88 and 96 nt DNA denaturation control fragments have been replaced (QDX2).
B1	One MLH1 probe (exon 19) and four MSH2 probes (exons 1, 1, 7 and 10) have been replaced. In addition, one extra MSH2 exon 1 probe, two extra EPCAM (formerly known as TACSTD1) probes and two extra control fragments at 100 and 105 nt have been included.
A2	One extra MSH2 exon 1 probe has been included.
A1	First release.

Implemented changes in the product description

Version D1-04 – 02 August 2018 (04)
- Product description restructured and adapted to a new template.
- Minor textual changes.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- Selected publications were updated using P003.
- Morocco and Israel were added as countries where product has IVD status.

Version D1-03 – 28 November 2017 (03)
- Intended use adjusted to clarify usage of product.
- Text on Binning DNA and Artificial Duplication DNA adjusted.
- Updated references using P003.
- Changed note on the 265 and 317 nt probes.
- Changed note on the 278 nt probe.
- Updated notes under Tables 1 and 2.
- Minor textual changes throughout the document.

Version D1-02 – 10 May 2017 (03)
- Product description restructured and adapted to a new template.

Version D1-01 – 18 November 2015 (02)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Various minor textual or layout changes.

Version 36 (02) – 20 April 2015
- Product description completely rewritten.

Version 35
- Product description adapted to a new lot (lot number added, new picture included).
- Minor textual changes.

Version 34
Product description completely rewritten.

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IVD	EUROPE* CE MOROCCO ISRAEL
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA). The product is for RUO in all other European countries.