Product Description
SALSA® MLPA® Probemix P248-B2 MLH1-MSH2 Confirmation

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, two reference probes have been replaced and three probe lengths have been adjusted. For complete product history see page 9.

Catalogue numbers:
- **P248-025R:** SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation, 25 reactions.
- **P248-050R:** SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation, 50 reactions.
- **P248-100R:** SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, 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.

Intended purpose
The SALSA MLPA probemix P248 MLH1-MSH2 Confirmation is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative 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, as initially determined using the SALSA MLPA probemix P003 MLH1/MSH2. P248 MLH1-MSH2 Confirmation cannot be used to verify deletions or duplications in *EPCAM* detected by P003 MLH1/MSH2.

Discordant results between the P248 MLH1-MSH2 Confirmation probemix and the P003 MLH1/MSH2 probemix 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 in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

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

Clinical background

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 an autosomal dominantly inherited syndrome that is 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). The estimated contribution of the different genes to Lynch syndrome is 15-40% for *MLH1*, 20-40% for *MSH2*, 12-35% for *MSH6*, 5-25% for *PMS2* and <10% for *EPCAM*. More information about Lynch syndrome is available on http://www.ncbi.nlm.nih.gov/books/NBK1211/.

Among the various defects in the *MLH1* and *MSH2* genes that have been found in patients, deletions and duplications of complete exons 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 containing 19 exons, and *MSH2* spans ~80 kb on chromosome 2p21 containing 16 exons. The *MLH1* LRG_216 is identical to GenBank NG_007109.2 and the *MSH2* LRG_218 is identical to GenBank NG_007110.2. Both LRGs are available at www.lrg-sequence.org/.

Transcript variants

For *MLH1*, multiple variants have been described. Transcript variant 1 represents the most abundant transcript, encoding the full length protein (NM_000249.4; 2494 nt; coding sequence 31-2301; http://www.ncbi.nlm.nih.gov/gene/4292). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 19.

For *MSH2*, two transcript variants have been described. Transcript variant 1 represents the longer transcript, encoding the longer isoform (NM_000251.3; 3115 nt; coding sequence 37-2841; http://www.ncbi.nlm.nih.gov/gene/4436). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 16.

Exon numbering

The *MLH1* and *MSH2* exon numbering used in this P248-B2 MLH1-MSH2 Confirmation product description is the exon numbering from the LRG_216 and LRG_218 sequences, respectively. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. 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

The SALSA MLPA Probemix P248-B2 MLH1-MSH2 Confirmation contains 49 MLPA probes with amplification products between 130 and 493 nucleotides (nt). This includes 21 probes for the *MLH1* gene, 17 probes for the *MSH2* gene and one probe located downstream of the *MSH2* gene. In addition, 10 reference probes are included that detect autosomal chromosomal locations. Almost all MLH1 and MSH2 probes included in probemix P003 MLH1/MSH2 have different ligation sites from those in P248, except for the 355 nt MSH2 exon 1 probe (02735-L20467). 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 the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.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 whole blood specimens, 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
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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 different 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 (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 NIBSC Institute in the U.K. provides a kit with five DNA samples (NIBSC code: 11/218-XXX) harbouring heterozygous *MLH1* or *MSH2* exon deletions or amplifications: http://www.nibsc.org/documents/ifu/11-218-xxx.pdf. These have been tested with this P248-B2 probemix at MRC Holland and can be used as positive control samples for deletions of *MSH2* exons 1-6, *MSH2* exon 7, *MSH2* exons 1-2, *MSH2* exon 1 and amplification of *MLH1* exon 13. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics
The diagnostic sensitivity of P248 is ~10%, as this is the total portion of Lynch syndrome which is caused by large rearrangements in *MLH1* and *MSH2*. Large deletions account for ~20-40% of variants in *MSH2* and ~10-20% of variants in *MLH1* (http://www.ncbi.nlm.nih.gov/books/NBK1211/). *MLH1* or *MSH2* duplications have been rarely reported, but can also be detected with this probemix. Note that the P248 MLH1-MSH2 Confirmation probemix does not contain probes for the detection of *MSH6*, *PMS2* and *EPCAM* 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: SNVs or other polymorphisms 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 should 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.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 expected results for the *MLH1* and *MSH2* 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 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	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software 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. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) 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: 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 (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. 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.

- Copy number changes detected by reference probes or flanking 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.

P248 specific notes:

- 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.
- A duplication of an internal part of *MLH1* or *MSH2* 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* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P248 MLH1-MSH2 Confirmation.
- 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

Detected copy number changes that are different from those detected with P003 MLH1/MSH2 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 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.

***MLH1/MSH2* mutation database**

We strongly encourage users to deposit positive results in the International Society for Gastrointestinal Hereditary Tumours (INSiGHT) and/or LOVD database (http://www.insight-group.org; http://www.lovd.nl). 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 SNVs and unusual results (e.g., a duplication of *MLH1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P248-B2 MLH1-MSH2 Confirmation

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*MLH1*	*MSH2*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 19551-L26105	2p		
136	**MSH2 probe** 20604-L28269			**Exon 10**
143	**MLH1 probe** 20605-L28740		**Exon 7**	
150	**MSH2 probe** 08661-L09622			**Exon 5**
154	**MLH1 probe** 07743-L09356		**Exon 2**	
160 ¥	**MSH2 probe** 21740-L32470			**Exon 7**
166 *	Reference probe 22048-L31008	1p		
172	**MLH1 probe** 01686-L28585		**Exon 1**	
178	**MSH2 probe** 00907-L28584			**Exon 3**
184	**MSH2 probe** 07760-L09355			**Exon 2**
190	Reference probe 20256-L23585	12q		
196	**MSH2 probe** 06344-L28739			**Exon 1**
202	**MLH1 probe** 08660-L28566		**Exon 18**	
208	**MLH1 probe** 07754-L28567		**Exon 13**	
214	**MLH1 probe** 07750-L28588		**Exon 9**	
220 ¥	**MLH1 probe** 20606-L32468		**Exon 11**	
227	**MSH2 probe** 07768-L28586			**Exon 12**
232	Reference probe 19652-L26275	4p		
238	**MLH1 probe** 07745-L28569		**Exon 4**	
244	**MSH2 probe** 07770-L28570			**Exon 15**
250	**MLH1 probe** 07742-L28571		**Exon 1**	
256	**MLH1 probe** 20607-L28272		**Exon 14**	
263	Reference probe 20636-L28350	17q		
269	**MSH2 probe** 20608-L28589			**Exon 6**
274	**MLH1 probe** 20609-L28274		**Exon 15**	
283	**MSH2 probe** 09140-L00579			**Exon 13**
292 ±	**MSH2 probe** 20610-L28275			**Exon 8**
301 ¥	**MLH1 probe** 21741-L32471		**Exon 3**	
310	**MSH2 probe** 07769-L28573			**Exon 14**
319	**MLH1 probe** 07747-L28574		**Exon 6**	
328	Reference probe 19756-L26539	9q		
337 « ¬	Flanking probe 08663-L13228			downstream
346	**MLH1 probe** 07751-L28583		**Exon 10**	
355 »	**MSH2 probe** 02735-L20467			**Exon 1**
362	**MLH1 probe** 07746-L28577		**Exon 5**	
373	Reference probe 19096-L24983	4q		
382	**MLH1 probe** 07758-L08431		**Exon 19**	
391	**MSH2 probe** 07766-L07496			**Exon 9**
400	**MLH1 probe** 07753-L08785		**Exon 12**	
409	**MSH2 probe** 07761-L07491			**Exon 4**
418	**MLH1 probe** 01012-L00574		**Exon 16**	
427	**MSH2 probe** 00920-L09358			**Exon 16**
436	**MLH1 probe** 01007-L00573		**Exon 17**	
445	Reference probe 18573-L24179	3q		
456	**MSH2 probe** 20611-L28276			**Exon 11**
463	**MLH1 probe** 11504-L28578		**Exon 1**	
472	Reference probe 19197-L25223	7p		
481	**MLH1 probe** 20612-L28277		**Exon 8**	
493 *	Reference probe 22813-L32176	22q		

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

* New in version B2.
¥ Changed in version B2. Minor alteration, no change in sequence detected.
± SNP rs546035846 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« Probe 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, especially of GC-rich regions.
» Detects the same sequence as the 148 nt probe in SALSA MLPA Probemix P003 MLH1/MSH2.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

Table 2. P248-B2 probes arranged according to chromosomal location

Table 2a. *MLH1* gene

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_000249.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
463	11504-L28578	Exon 1	516 nt before exon 1	CCACCACCAAAT-AACGCTGGGTCC	0.5 kb
172	01686-L28585	Exon 1	18-19	TTCCTTGGCTCT-TCTGGCGCCAAA	0.1 kb
		start codon	*31-33 (Exon 1)*		
250	07742-L28571	Exon 1	2 nt after exon 1	TTGAGAACTGGT-ACGGAGGGAGTC	2.7 kb
154	07743-L09356	Exon 2	230 nt before exon 2	AGAGGAACAGAA-ATAGTGCTGAAT	4.7 kb
301	21741-L32471	Exon 3	8 nt after exon 3	TGAGGTAAGCTA-AAGATTCAAGAA	3.4 kb
238	07745-L28569	Exon 4	398-397 reverse	TGTATGCACACT-TTCCATCAGCTG	2.6 kb
362	07746-L28577	Exon 5	14 nt after exon 5 reverse	TTTACTCTCCCA-TGTACCATTCTT	1.8 kb
319	07747-L28574	Exon 6	530-531	AAAAGCTTTAAA-AAATCCAAGTGA	3.0 kb
143	20605-L28740	Exon 7	51 nt after exon 7	AAAGAAAAAAGG-GGATTTTTAATA	0.3 kb
481	20612-L28277	Exon 8	111 nt after exon 8 reverse	TGTATTTGACTA-AAGCAAACTCTT	2.4 kb
214	07750-L28588	Exon 9	57 nt after exon 9 reverse	ATGGTCCCATAA-AATTCCCTGTGG	3.0 kb
346	07751-L28583	Exon 10	887-888	CTATTTGCCCAA-AAACACACACCC	2.9 kb
220	20606-L32468	Exon 11	33 nt after exon 11	CTACTTCTCTGG-GGCCTTTGAAAT	5.2 kb
400	07753-L08785	Exon 12	1120-1119 reverse	GGTCAGACTTGT-TGTGGATTTAAC	3.2 kb
208	07754-L28567	Exon 13	1547-1548	CCTCACTAGTGT-TTTGAGTCTCCA	11.4 kb
256	20607-L28272	Exon 14	26 nt after exon 14	TGTGTGAACAAG-CAGAGCTACTAC	2.1 kb
274	20609-L28274	Exon 15	58 nt after exon 15	TGAAAATAGTAG-CTCTCCACTAAT	5.2 kb
418	01012-L00574	Exon 16	1833-1834	ACAGAGGAAGAT-GGTCCCAAAGAA	1.0 kb
436	01007-L00573	Exon 17	1966-1967	TGATTGACAACT-ATGTGCCCCCTT	0.5 kb
202	08660-L28566	Exon 18	17 nt after exon 18 reverse	TGGGGTGCCAGT-GTGCATCACCAC	1.6 kb
		stop codon	*2299-2301 (Exon 19)*		
382	07758-L08431	Exon 19	2302-2301 reverse	AAATAACCATAT-TTAACACCTCTC	

Table 2b. *MSH2* gene

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_000251.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
196	06344-L28739	Exon 1	233 nt before exon 1 reverse	GACGCGCATCCT-TAGTAGAGCTCC	0.1 kb
355 »	02735-L20467	Exon 1	156 nt before exon 1	GCGTGCGCGGGA-AGCTGGGCCGCG	5.4 kb
		start codon	*37-39 (Exon 1)*		
184	07760-L09355	Exon 2	269-270	TCTGCAGAGTGT-TGTGCTTAGTAA	1.8 kb
178	00907-L28584	Exon 3	564-565	CTAGGACTGTGT-GAATTCCCTGAT	2.3 kb
409	07761-L07491	Exon 4	809-808 reverse	CCATTTCTGGCA-ATACAGCACTAT	1.8 kb
150	08661-L09622	Exon 5	932-933	CTTCAGCCAGTA-TATGAAATTGGA	1.9 kb
269	20608-L28589	Exon 6	59 nt before exon 6	GTTTTTCATGGC-GTAGTAAGGTTT	13.5 kb

Length (nt)	SALSA MLPA probe	Gene exon[a]	Ligation site NM_000251.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
160	21740-L32470	Exon 7	1148-1149	AGAAGATGCAGA-ATTGAGGCAGAC	16.0 kb
292 ±	20610-L28275	Exon 8	93 nt after exon 8	TTTTAGTATCTA-ATTGTAGCACCT	17.4 kb
391	07766-L07496	Exon 9	1523-1522 reverse	CTGCACTTATTA-ATGTTGACTGCA	3.6 kb
136	20604-L28269	Exon 10	1570-1569 reverse	GGAATCCAGTTT-AATCTGTTTGCC	4.4 kb
456	20611-L28276	Exon 11	43 nt after exon 11	TGTCACCTGGCT-TTTGGTAACAGA	4.0 kb
227	07768-L28586	Exon 12	1822-1823	TGCAGACACTCA-ATGATGTGTTAG	1.4 kb
283	09140-L00579	Exon 13	2161-2162	TGGACTGCATCT-TAGCCCGAGTAG	2.0 kb
310	07769-L28573	Exon 14	2431-2432	CAACTGTTAATA-ATCTACATGTCA	2.2 kb
244	07770-L28570	Exon 15	2520-2519 reverse	TCTGCAACATGA-ATCCCAAAACTT	2.1 kb
427	00920-L09358	Exon 16	2714-2715	CAAGGTGAAACA-AATGCCCTTTAC	38.5 kb
		stop codon	*2839-2841 (Exon 16)*		
337 « ¬	08663-L13228	*KCNK12*		CCTGCTCGGCGT-GTGCTGCATTTA	

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

± SNP rs546035846 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« Probe 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, especially of GC-rich regions.
» Detects the same sequence as the 148 nt probe in SALSA MLPA Probemix P003 MLH1/MSH2.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

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

Related SALSA MLPA probemixes

Condition		Gene	Probemix
Lynch syndrome (HNPCC)		*MLH1*	P003 MLH1/MSH2 P248 MLH1-MSH2 Confirmation ME011 Mismatch Repair Genes ME042 CIMP
		MSH2	P003 MLH1/MSH2 P248 MLH1-MSH2 Confirmation ME011 Mismatch Repair Genes
		MSH6	P072 MSH6-MUTYH ME011 Mismatch Repair Genes
		PMS2	P008 PMS2 ME011 Mismatch Repair Genes
		EPCAM	P003 MLH1/MSH2 P072 MSH6-MUTYH ME011 Mismatch Repair Genes
Polyposis syndrome	MAP	*MUTYH*	P378 MUTYH P043 APC P072 MSH6-MUTYH
	AFAP	*APC*	P043 APC
	FAP	*APC*	P043 APC

References

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P248 product history	
Version	*Modification*
B2	Two reference probes have been replaced and three probe lengths have been adjusted.
B1	Four MSH2 probes and six MLH1 probes have been replaced. One MLH1 exon 1 probe and the downstream MLH1 flanking probe have been removed. Reference probes have been replaced and six new added. Control Fragments adjusted (QDX2).
A2	Two extra control fragments at 100-105 nt have been included.
A1	First release.

Implemented changes in the product description
Version B2-03– 24 February 2023 (04P) - Clinical background updated according to the most recent literature. - SNP warning added to Table 1 and 2 for the 292 nt probe. - Minor textual or layout changes. Version B2-02– 29 October 2021 (04P) - Performance characteristics updated in line with the P003 product description. - Minor textual and layout changes. Version B2-01 – 24 February 2021 (04P) - Product description rewritten and adapted to a new template. - P248-B2 is now CE marked. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *MLH1* and *MSH2* genes updated according to new version of the NM_ reference sequence.

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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

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