Product Description
SALSA® MLPA® Probemix P043-E1 APC

To be used with the MLPA General Protocol.

Version E1
For complete product history see page 14.

Catalogue numbers:
- **P043-025R:** SALSA MLPA Probemix P043 APC, 25 reactions.
- **P043-050R:** SALSA MLPA Probemix P043 APC, 50 reactions.
- **P043-100R:** SALSA MLPA Probemix P043 APC, 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 P043 APC 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 *APC* and *MUTYH* genes, and duplications in the upstream region of the *GREM1* gene in order to confirm a potential cause for and clinical diagnosis of familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), or hereditary mixed polyposis syndrome (HMPS1), respectively. 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. P043 APC can also be used for molecular genetic testing of at-risk family members. This assay is for use with genomic DNA extracted from human peripheral whole blood specimens.

Copy number variations (CNVs) detected with P043 APC 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 *APC* and *MUTYH* genes 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. FAP has a high incidence of mosaicism and mosaic *APC* mutations may not be detectable in blood.

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, Coffalyser.Net analysis software, and SALSA Binning DNA SD022.

Clinical background

Germline defects in the *APC* gene are the most frequent cause of a hereditary predisposition to polyposis colon cancer, which can present as familial adenomatous polyposis (FAP), attenuated FAP (AFAP), or gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS). In addition, acquired mutations in the *APC* gene are an initiating event for sporadic colorectal tumour development. Most FAP patients develop adenomatous colonic polyps in the first two decades of life and in untreated individuals this progresses into colonic cancer at an average age of 39 years. *APC*-related colorectal cancer (CRC) is a dominant trait. Approximately 4% of (A)FAP patients have a somatic mosaic *APC* mutation, which increases to ~20% when only *de novo* cases are considered. More information on APC-associated polyposis is available at http://www.ncbi.nlm.nih.gov/books/NBK1345/.

Mutations in the *MUTYH* gene also result in a hereditary predisposition to colon and gastric cancer, which is referred to as MAP. In contrast to the *APC*-associated disease FAP, MAP is an autosomal recessive trait and is considered less severe: polyps do not appear until adulthood and are less numerous than those found in patients with *APC* gene mutations. Nevertheless, phenotypes of *APC*- and *MUTYH*-related CRC partly overlap. Therefore, six probes for the *MUTYH* gene are included in this P043-E1 APC probemix, two of which will only generate a signal when the common c.536A>G (p.Tyr179Cys) or c.1187G>A (p.Gly396Asp) mutations are present. The c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp) variants are carried by ~1%-2% of the general population and account for ≥90% of all *MUTYH* pathogenic variants in northern European populations. Up to 70% of MAP patients harbours at least one of these variants (Aretz *et al.* 2013). Since the *MUTYH* gene is small (11 kilobases (kb)), the four copy number detection MLPA probes are expected to detect a substantial part of *MUTYH* copy number changes. For instance, the most frequent CNV in *MUTYH* – a deletion of exon 4-16 that is reported in multiple patients (Castillejo *et al*. 2014) – can be detected with two probes in this probemix. More information on MAP is available at http://www.ncbi.nlm.nih.gov/books/NBK107219/. For complete exon coverage of *MUTYH* the SALSA MLPA Probemix P378 MUTYH is available.

A recurrent duplication of ~40 kb directly upstream of the *GREM1* gene is known to lead to HMPS1. Patients with HMPS1 have a predisposition for developing CRC (Jaeger *et al.* 2012). Presence of this duplication is predicted to cause reduced bone morphogenetic protein (BMP) pathway activity, a mechanism that underlies tumorigenesis in juvenile polyposis of the large bowel. Several additional duplications in the *GREM1* upstream region have been found: *e.g.* a duplication of the upstream region and the whole *GREM1* gene of ~57 kb has been described in one patient with sigmoid colon carcinoma (Venkatachalam *et al.* 2011); a duplication of ~16 kb has been described in members of a family presenting with atypical FAP (Rohlin *et al.* 2016); and a duplication of ~24 kb in a patient with multiple colon polyps has been reported (McKenna *et al.* 2019). Two probes for the relevant region directly upstream of *GREM1* are included in this probemix; both probes detect the 40 kb, 57 kb and 24 kb duplications and the 16 kb duplication can be detected by one of these probes (probe number 21272-L23310 at 217 nucleotides (nt); see Table 2 for more details). For extended coverage of the *GREM1* region the SALSA MLPA Probemix P378 MUTYH is available. More information on HMPS1 is available at http://omim.org/entry/601228.

Among the various defects in the *APC* and *MUTYH* 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 *APC* and *MUTYH* genes. It is expected that 8-12% of all *APC* mutations in most populations are large rearrangements that can be detected with this MLPA probemix (Jarry *et al.* 2011; Kerr *et al.* 2013). Of note, several clinically relevant rearrangements of only the *APC* promoter region are reported (Snow *et al.* 2014; Kadiyska *et al.* 2014; Kalbfleisch *et al.* 2015; Marabelli *et al.* 2017), all of which can be detected with this probemix (see Table 2 for more details).

Gene structure
The *APC* gene spans ~108 kb on chromosome 5q22.2, 69 Mb from the q-telomere. *APC* contains 18 exons (note that there are different *APC* transcript variants and they do not all contain 18 exons; see below for more information). The *APC* LRG_130 is available at www.lrg-sequence.org and is identical to GenBank NG_008481.4.

The *MUTYH* gene spans ~11 kb on chromosome 1p34.1. *MUTYH* contains 16 exons. The *MUTYH* LRG_220 is available at www.lrg-sequence.org and is identical to GenBank NG_008189.1.

The *GREM1* gene spans ~27 kb on chromosome 15q13.3. *GREM1* contains 2 exons. The *GREM1* LRG_1365 is available at www.lrg-sequence.org and is identical to GenBank NG_033791.2. This P043-E1 APC probemix only contains two probes targeting sequences 5-8 kb upstream of *GREM1*.

Transcript variants
For *APC,* multiple transcript variants have been described. Transcript variant 3 (NM_000038.6; 10704 nt; coding sequence (CDS) 60-8591) represents the longest transcript, encodes the long isoform b and it is also most often used in scientific literature to annotate *APC* mutations. In Table 2a, the ligation sites of the *APC* MLPA probes are indicated according to this transcript, which includes 16 of the 18 *APC* exons. NM_000038.6 lacks exons 1 and 3, which are therefore indicated with other transcripts in Table 2a. Please note, that in tables 1 and 2a next to the LRG_130 exon numbering, also the exon numbering from the MANE Select transcript NM_000038.6 is indicated between brackets.
Transcript variant 2 (NM_001127510.3; 10812 nt; CDS 168-8699) also encodes the long isoform b, but includes exon 3, and lacks exon 1. Transcript variant 1 (NM_001127511.3; 10811 nt; CDS 221-8698) includes exon 1, but it lacks exon 2 and 3, and encodes the shorter isoform a. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/324. *APC* exon 1 is also referred to as the *Promoter 1B region* and exon 2 as the *Promoter 1A region* in literature.

For *MUTYH,* multiple variants have been described. Transcript variant alpha5 (NM_001128425.2; 1900 nt; CDS 187-1836) represents the longest transcript and encodes the long isoform 5. In Table 2b, the ligation sites of the *MUTYH* MLPA probes are indicated according to this sequence. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/4595.

For *GREM1,* multiple variants have been described. Transcript variant 1 (NM_013372.7; 14575 nt; CDS 160-714) represents the longest transcript and encodes isoform 1. In Table 2c, the ligation sites of the *GREM1* MLPA probes are indicated in relation to this sequence. More information can be found on the NCBI gene page: https://www.ncbi.nlm.nih.gov/gene/26585.

Exon numbering
The *APC* exon numbering used in this P043-E1 APC product description is the exon numbering from the LRG_130 sequence. Please note that the exon numbering from the MANE Select transcript NM_000038.6 is indicated between brackets. For *MUTYH* the exon numbering from the LRG_220 was used and for *GREM1* the LRG_1365. The NM_ sequences that were used for determining probe ligation sites do not always contain all exons in 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. Please note that exon numbering for the same genes might be different from literature and other MRC Holland product descriptions, where other resources used for exon numbering are indicated.

Probemix content
The SALSA MLPA Probemix P043-E1 APC contains 45 MLPA probes with amplification products between 130 and 483 nt. This includes 29 probes for the *APC* gene, four probes for the *MUTYH* gene, and two probes for the region upstream of *GREM1*. This probemix also contains two probes specific for the c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp) *MUTYH* mutations, which will only generate a signal when the mutation is present. In addition, eight 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 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. Note that the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.

Required specimens
Extracted DNA from 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
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. Sample ID numbers from the Coriell Institute described in the table below have been tested with this P043-E1 Probemix at MRC Holland and can be used as positive control samples. Note that the two *MUTYH* mutation-specific probes are only intended to determine the presence of the mutation and should not be used to determine zygosity. The quality of cell lines can change; therefore, samples should be validated before use.

Coriell sample ID	Affected target genes	Expected result
NA14234	*APC*	Heterozygous deletion of the complete *APC* gene
HG01918	*MUTYH*	Positive for the *MUTYH* c.536A>G (p.Tyr179Cys) mutation

Coriell sample ID	Affected target genes	Expected result
HG00097; HG01095; HG01519; HG01685; HG02224; NA19789; NA20522; NA20759	*MUTYH*	Positive for the *MUTYH* c.1187G>A (p.Gly396Asp) mutation
NA03184	*GREM1* region	Heterozygous duplication detected by both probes present in Table 2c

SALSA Binning DNA SD022

The SALSA Binning DNA SD022 provided with this probemix can be used for binning of all probes, including the two *MUTYH* mutation-specific probes: the 188 nt probe 18416-SP0654-L29811, detecting the c.536A>G (p.Tyr179Cys) mutation; and the 193 nt probe 21267-SP0655-L23442, detecting the c.1187G>A (p.Gly396Asp) mutation. The Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target sequence detected by the above mentioned mutation-specific probes. Inclusion of one reaction with 5 µl SD022 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 signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SALSA Binning DNA SD022 product description, available online: www.mrcholland.com.

Performance characteristics

The majority of FAP patients has a point mutation in the *APC* gene, which is easily detectable by sequence analysis. It is estimated that approximately 8-12% of all FAP patients have large rearrangements in the *APC* gene, either involving part of the gene or the whole gene and surrounding regions, all of which can be detected with the P043-E1 APC probemix. When MLPA is used in addition to sequence analysis of the *APC* gene, the detection rate therefore increases with ~10%.

Almost all MAP patients have biallelic point mutations in the *MUTYH* gene. Deletions in this gene have rarely been described: the percentage of cases explained by large deletions/duplications is estimated to be <1%. The two most common *MUTYH* point mutations, c.536A>G (p.Tyr179Cys) and c.1187G>A (p.Gly396Asp), account for ≥90% of all *MUTYH* pathogenic variants in northern European populations. Up to 70% of MAP patients harbors at least one of these variants. The presence of these two mutations can be detected by this probemix. Based on the populations tested in scientific literature the diagnostic sensitivity of the P043-E1 APC Probemix for MAP is estimated at ~70%.

The only known cause of HMPS1 are large duplications in the upstream region of *GREM1* that may, or may not, include the *GREM1* gene. The P043 probemix contains two probes that target the relevant region, which enables detection of all known duplications in this region (see Table 2c for more details). Therefore the diagnostic sensitivity of the P043-E1 APC Probemix for HMPS1 is 100%.

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 all copy number probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion) or 3 (heterozygous duplication). In rare cases, copy numbers of 0 (homozygous deletion) or 4 (heterozygous triplication/homozygous duplication) can be obtained. The standard deviation of each individual probe (with exception of the mutation-specific probes) 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 copy number 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.

The above mentioned FR values do not apply to the mutations-specific probes. The peaks of the mutation-specific probes are expected to be absent in the majority of samples tested and therefore their standard deviation cannot be determined. Clear signal (at least 10% of the median peak height of all reference probes in that sample) for one of these probes indicates that the mutation is present.

- 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.

P043 specific notes

- Two probes are present for the *APC* promoter region 1B (exon 1 in NM_001127511.1). Promoter 1B deletions have been linked to a hereditary predisposition to adenomatous polyposis by multiple studies (Rohlin *et al.* 2011; Kadiyska *et al.* 2014; Snow *et al.* 2015; Marabelli *et al.* 2017; Araujo *et al.* 2019), and so have promoter 1B duplications (Khan *et al.* 2017). Furthermore, Snow *et al.* (2015) established that a deletion of the promoter region 1B is a potential founder mutation in the U.S. population, as the deletion was reported in seven North American FAP families.
- Two probes are present that detect the wildtype sequence at the site of two frequent *APC* point mutations:
  1) The presence of the c.3183_3187delACAAA (rs587779352; p.Lys1061_Gln1062insTer) mutation will result in a decreased probe signal of the probe at 419 nt. In literature this mutation is sometimes also referred to as the 'codon 1061 mutation'.
  2) The presence of the c.3927_3931delAAAGA (rs121913224; p.Glu1309fs) mutation will result in a decreased probe signal of the probe at 427 nt.

  A reduced signal of these probes can be due to the presence of the aforementioned mutations, but can also be due to a genomic deletion. Note that other variants near or on the ligation site can also cause a lowered signal.
- The two *MUTYH* mutation-specific probes are intended to determine the presence (or absence) of the mutation.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *APC* gene are small (point) mutations that, except for the two mutations mentioned above, will not be detected by the SALSA MLPA Probemix P043 APC. The presence of the two most common point mutations in the *MUTYH* gene among Europeans can be detected, but other point mutations in *MUTYH* cannot.
- 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 as well as the two common *MUTYH* point mutations always require confirmation by another method. Because the two *MUTYH* mutation-specific probes are only intended to determine the presence of the mutation, positive results obtained for either of these probes need to be confirmed by sequence analysis to determine the zygosity of the mutation.

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.

Mutation databases

We strongly encourage users to deposit positive results in the *APC* LOVD mutation database: https://databases.lovd.nl/shared/genes/APC, and/or in the *MUTYH* LOVD mutation database: https://databases.lovd.nl/shared/genes/MUTYH. 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 *APC* exons 8 and 10 but not exon 9) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P043-E1 APC

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*APC*^	*MUTYH*	*GREM1*
64-105	Control fragments – see table in probemix content section for more information				
130	Reference probe 00797-L00463	5q			
136	**MUTYH probe** 21270-L28956			**Exon 11**	
142	**APC probe** 01532-L01743		**Exon 2 [1]**		
148	**MUTYH probe** 21271-L29806			**Exon 16**	
154	**APC probe** 11988-L29807		**Exon 2 [1]**		
160	Reference probe 13447-L29808	17p			
166	**APC probe** 18176-L29809		**Exon 18 [16]**		
172	**APC probe** 01534-L29810		**Exon 3**		
178	**APC probe** 01535-L19321		**Exon 4 [2]**		
188 §	**MUTYH probe** 18416-SP0654-L29811			**c.536A>G mutation-specific**	
193 §	**MUTYH probe** 21267-SP0655-L23442			**c.1187G>A mutation-specific**	
202	**APC probe** 01536-L29812		**Exon 5 [3]**		
209	**APC probe** 01537-L29813		**Exon 6 [4]**		
217	**GREM1 probe** 21272-L23310				**8.4 kb before exon 1**
224 ±	**APC probe** 01538-L29814		**Exon 7 [5]**		
229	**APC probe** 01539-L01337		**Exon 8 [6]**		
238	Reference probe 10089-L10513	8q			
246	**APC probe** 01540-L00983		**Exon 9 [7]**		
253	**MUTYH probe** 02110-L04024			**Exon 1**	
260	**APC probe** 15274-L19324		**Exon 1 [up]**		
267	**APC probe** 01541-L19325		**Exon 10 [8]**		
274 Δ	**APC probe** 15276-L19326		**Exon 1 [up]**		
282	**APC probe** 01542-L19327		**Exon 11 [9]**		
289	**APC probe** 11990-L19328		**Exon 12 [10]**		
297	Reference probe 04355-L19329	7q			
306	**APC probe** 01544-L00987		**Exon 13 [11]**		
312	**APC probe** 01545-L01338		**Exon 14 [12]**		
328	**APC probe** 01774-L01340		**Exon 15 [13]**		
337	**APC probe** 01700-L01341		**Exon 16 [14]**		
346	**MUTYH probe** 14912-L19331			**Exon 2**	
357	**APC probe** 21269-L29815		**Exon 18 [16]**		
365	**APC probe** 01548-L20445		**Exon 17 [15]**		
373	Reference probe 15525-L17380	16q			
382	**APC probe** 21268-L29625		**Exon 18 [16]**		
391	**APC probe** 12035-L29816		**Exon 18 [16]**		
400	Reference probe 08871-L08927	1p			
409 ◍	**APC probe** 03323-L02526		**Intron 13 [11]**		
419 ∞	**APC probe** 03324-L29817		**Exon 18 [16]**		
427 ∞	**APC probe** 03325-L02528		**Exon 18 [16]**		
436	**APC probe** 18178-L24041		**Exon 18 [16]**		
445	Reference probe 17736-L21863	6q			
454	**APC probe** 18175-L22760		**Exon 18 [16]**		
463	**APC probe** 18177-L22762		**Exon 18 [16]**		
474	**GREM1 probe** 21273-L23308				**5.6 kb before exon 1**
483	Reference probe 12761-L29818	4q			

[a] See section Exon numbering on page 3 for more information.

^ Ligation sites for *APC* are indicated according to MANE Select transcript NM_000038.6, and the exon numbering is according to LRG_130. Exon numbering according to MANE Select transcript NM_000038.6 is indicated between brackets.
§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. Both mutation-specific probes consist of three parts and have two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. Masking of the mutation-specific signal due to another mutation or SNP in the probe target can only occur when both are present on the same allele.
∞ Wild type sequence detected. The presence of the *APC* c.3183_3187delACAAA (rs587779352; p.Lys1061_Gln1062insTer) mutation will result in a decreased probe signal of the probe at 419 nt. The presence of the *APC* c.3927_3931delAAAGA (rs121913224; p.Glu1309fs) will result in a decreased probe signal of the probe at 427 nt. A reduced signal of these probes can also be due to other variants near the ligation site or to a deletion. A positive result must be confirmed by another method.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
ⓞ This sequence is located in the *APC* "exon 10a" of GenBank file Z49221.1, and is present in alternative transcripts NM_001354896.2 (ligation site 1493-1494) and NM_001354900.2 (ligation site 1340-1341). This alternative exon is not included in the *APC* LRG_130. Deletions or duplications detected only by this probe have unknown clinical consequence.
± SNP rs116020626 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

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

Table 2. P043-E1 probes arranged according to chromosomal location

Table 2a. *APC*

Length (nt)	SALSA MLPA probe	*APC*^ exon[a]	Ligation site NM_000038.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
260 +	15274-L19324	Exon 1 [up]	12-13 (NM_001127511.3)	GCATTGTAGTCT-TCCCACCTCCCA	0.4 kb
274 + Δ	15276-L19326	Exon 1 [up]	366-367 (NM_001127511.3)	TACTTCTGGCCA-CTGGGCGAGCGT	29.7 kb
142 +	01532-L01743	Exon 2 [1]	324 nt before exon 2 [1]	CTCCGGCATCTT-GTGCTAATCCTT	0.3 kb
154 +	11988-L29807	Exon 2 [1]	23 nt before exon 2 [1]	CATGTGGCTGTA-TTGGTGCAGCCC	0.5 kb
172	01534-L29810	Exon 3	91-92 (NM_001127510.3)	ACCGCTGCAGAT-GGCTGATGTGAA	16.5 kb
		start codon	*60-62 (exon 4 [2])*		
178	01535-L19321	Exon 4 [2]	76-77	TGCAGCTTCATA-TGATCAGTTGTT	11.5 kb
202	01536-L29812	Exon 5 [3]	242-243	GAAGCTATGGCT-TCTTCTGGACAG	0.9 kb
209	01537-L29813	Exon 6 [4]	365-366	CGGGAAGGATCT-GTATCAAGCCGT	8.4 kb
224 ±	01538-L29814	Exon 7 [5]	510-511	ATCTTGACAAAG-AAGAAAAGGAAA	5.2 kb
229	01539-L01337	Exon 8 [6]	662-663	GTTGCGATGGAA-GAACAACTAGGT	11.6 kb
246	01540-L00983	Exon 9 [7]	731-732	ATTCAGCAAATC-GAAAAGGACATA	8.8 kb
267	01541-L19325	Exon 10 [8]	815-816	AAGCATGAAACC-GGCTCACATGAT	14.2 kb
282	01542-L19327	Exon 11 [9]	923-924	GACCATGAAACA-GCCAGTGTTTTG	3.8 kb
289	11990-L19328	Exon 12 [10]	1339-1340	GCAGGAAGCTCA-TGAACCAGGCAT	2.6 kb
306	01544-L00987	Exon 13 [11]	1420-1421	GTGTGTTCTAAT-GAAACTTTCATT	1.4 kb
409 ⓞ	03323-L02526	Intron 13 [11]	1341 nt after exon 13 [11]	CGGGGCATTTCA-TCACAGGAGCTA	3.8 kb
312	01545-L01338	Exon 14 [12]	1494-1495	TTGCAGAATTAT-TGCAAGTGGACT	0.8 kb
328	01774-L01340	Exon 15 [13]	1654-1653, reverse	CAGATTTTAGTT-GGGCCACAAGTG	0.9 kb
337	01700-L01341	Exon 16 [14]	1727-1728	TGGCGAGCAGAT-GTAAATAGTAAA	6.2 kb
365	01548-L20445	Exon 17 [15]	1904-1905	GCACTTGCATTT-TTGGTTGGCACT	2.7 kb
382	21268-L29625	Exon 18 [16]	2236-2237	TGCTGCAGCTTT-AAGGAATCTCAT	1.0 kb
419 ∞	03324-L29817	Exon 18 [16]	3241-3242	AGATGAAATAAA-ACAAAGTGAGCA	0.7 kb
427 ∞	03325-L02528	Exon 18 [16]	3987-3986, reverse	AGTTCCAATCTT-TTCTTTTATTTC	0.9 kb
357	21269-L29815	Exon 18 [16]	4898-4899	AGTCAGCTGCCT-GTGTACAAACTT	0.7 kb
454	18175-L22760	Exon 18 [16]	5613-5614	CTATTGAAGGAA-CTCCTTACTGTT	0.7 kb
166	18176-L29809	Exon 18 [16]	6300-6301	CACTTGATTTGA-AAGATATACAGA	0.7 kb

Length (nt)	SALSA MLPA probe	*APC*^ exon[a]	Ligation site NM_000038.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
463	18177-L22762	Exon 18 [16]	7010-7011	CAGCAACCATTA-AGTAGACCTATA	0.7 kb
436	18178-L24041	Exon 18 [16]	7718-7719	GAGCACAGCAAA-CATTCATCATCC	0.8 kb
391	12035-L29816	Exon 18 [16]	8493-8494	CAGTGAATAACA-ACACAAAGAAGC	
		stop codon	*8589-8591 (exon 18 [16])*		

Table 2b. *MUTYH*

Length (nt)	SALSA MLPA probe	*MUTYH* exon[a]	Ligation site NM_001128425.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
253	02110-L04024	Exon 1	162-163	GGAGCTGAAACT-GCGCCATCGTCA	5.9 kb
		start codon	*187-189 (exon 1)*		
346	14912-L19331	Exon 2	316-317	CTAAGAACAACA-GTCAGGCCAAGC	1.6 kb
188 §	18416-SP0654-L29811	c.536A>G (p.Tyr179Cys) in exon 7	722-721 & 694-693, reverse	CACGAGAATAG**C**-28 nt spanning oligo-CTCCTGTGGGTA	0.7 kb
136	21270-L28956	Exon 11	1129-1128, reverse	TAAGAGCTGTTC-CTGCTCCACCTG	0.6 kb
193 §	21267-SP0655-L23442	c.1187G>A (p.Gly396Asp) in exon 13	1373-1374 & 1408-1409	CTCCCTCTCAG**A**-35 nt spanning oligo-CCTGGGAGCCCT	2.2 kb
148	21271-L29806	Exon 16	1823-1822, reverse	ACTGGGCTGCAC-TGTTGAGGCTGT	
		stop codon	*1834-1836 (exon 16)*		

Table 2c. *GREM1* region

Length (nt)	SALSA MLPA probe	*GREM1* region[a]	Ligation site NM_013372.7	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
217 ∫	21272-L23310	Upstream	8.4 kb before exon 1	AGAAACAAACAC-TGCAGGCAAGGT	2.9 kb
474	21273-L23308	Upstream	5.6 kb before exon 1	ACAGGTTACCCT-GTCTGCAGACAA	

[a] See section Exon numbering on page 3 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.

^ Ligation sites for *APC* are indicated according to MANE Select transcript NM_000038.6, and the exon numbering is according to LRG_130. Exon numbering according to MANE Select transcript NM_000038.6 is indicated between brackets.
§ Mutation-specific probe. This probe will only generate a signal when the indicated mutation is present. Both mutation-specific probes consist of three parts and have two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. Masking of the mutation-specific signal due to another mutation or SNP in the probe target can only occur when both are present on the same allele.
∞ Wild type sequence detected. The presence of the *APC* c.3183_3187delACAAA (rs587779352; p.Lys1061_Gln1062insTer) mutation will result in a decreased probe signal of the probe at 419 nt. The presence of the *APC* c.3927_3931delAAAGA (rs121913224; p.Glu1309fs) will result in a decreased probe signal of the probe at 427 nt. A reduced signal of these probes can also be due to other variants near the ligation site or to a deletion. A positive result must be confirmed by another method.
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
◎ This sequence is located in the *APC* "exon 10a" of GenBank file Z49221.1, and is present in alternative transcripts NM_001354896.2 (ligation site 1493-1494) and NM_001354900.2 (ligation site 1340-1341). This alternative exon is not included in the *APC* LRG_130. Deletions or duplications detected only by this probe have unknown clinical consequence.
+ *APC* exon 1 is also referred to as *Promoter region 1B* and exon 2 is also referred to as *Promoter region 1A*. Copy number changes in these promoter regions can be detected with these probes.
∫ The 16 kb duplication reported in Rohlin *et al.* 2015 is detected by this probe only. The other duplications in this region, mentioned earlier in this product description, affect both *GREM1* upstream probes.
± SNP rs116020626 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. 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	SALSA MLPA 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
	FAP	*APC*	P043 APC
	AFAP	*APC*	P043 APC

References

- Araujo LF *et al.* (2019). Molecular basis of familial adenomatous polyposis in the southeast of Brazil: identification of six novel mutations. *Int J Biol Markers*.1724600818814462.
- Aretz S *et al.* (2013). Clinical utility gene card for: MUTYH-associated polyposis (MAP), autosomal recessive colorectal adenomatous polyposis, multiple colorectal adenomas, multiple adenomatous polyps (MAP) - update 2012. *Eur J Hum Genet*. 21.
- Castillejo A *et al*. (2014). Prevalence of germline MUTYH mutations among Lynch-like syndrome patients. *Eur J Cancer*. 50:2241-2250.
- Farrington SM *et al.* (2005). Germline susceptibility to colorectal cancer due to base-excision repair gene defects. *Am J Hum Genet.* 77(1):112-119.
- Jaeger E *et al.* (2012). Hereditary mixed polyposis syndrome is caused by a 40-kb upstream duplication that leads to increased and ectopic expression of the BMP antagonist GREM1. *Nature Genet*. 44: 699-703.
- Jarry J *et al.* (2011). A survey of APC mutations in Quebec. *Fam Cancer.* 10(4):659-665.
- Kadiyska T *et al.* (2014). APC promoter 1B deletion in familial polyposis—implications for mutation-negative families. *Clin Genet.* 85(5):452-457.
- Kalbfleisch T *et al.* (2015). Characterization of an APC Promoter 1B deletion in a Patient Diagnosed with Familial Adenomatous Polyposis via Whole Genome Shotgun Sequencing. *F1000Res.* 4:170.
- Khan N *et al.* (2017). Novel mutations and phenotypic associations identified through APC, MUTYH, NTHL1, POLD1, POLE gene analysis in Indian Familial Adenomatous Polyposis cohort. *Sci Rep*. 7:2214.
- Kerr SE *et al.* (2013). APC germline mutations in individuals being evaluated for familial adenomatous polyposis: a review of the Mayo Clinic experience with 1591 consecutive tests. *J Mol Diagn*. 15(1):31-43.
- Marabelli M *et al.* (2017). A novel APC promoter 1B deletion shows a founder effect in Italian patients with classical familial adenomatous polyposis phenotype. *Genes Chromosomes Cancer*. 56:846-854.
- McKenna DB *et al.* (2019). Identification of a novel GREM1 duplication in a patient with multiple colon polyps. *Fam Cancer*. 18:63-66.
- Rohlin A *et al.* (2011). Inactivation of promoter 1B of APC causes partial gene silencing: evidence for a significant role of the promoter in regulation and causative of familial adenomatous polyposis. *Oncogene*. 30(50):4977-4989.

- Rohlin A *et al.* (2016). GREM1 and POLE variants in hereditary colorectal cancer syndromes. *Genes Chromosomes Cancer*. 55(1):95-106.
- 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.
- Snow AK *et al.* (2015). APC promoter 1B deletion in seven American families with familial adenomatous polyposis. *Clin Genet*. 88:360-365.
- 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.
- Venkatachalam R et a. (2011). Identification of candidate predisposing copy number variants in familial and early-onset colorectal cancer patients. *Int J Cancer*. 129(7):1635-1642.

Selected publications using SALSA MLPA Probemix P043 APC

- Araujo LF *et al.* (2019). Molecular basis of familial adenomatous polyposis in the southeast of Brazil: identification of six novel mutations. *Int J Biol Markers*.1724600818814462.
- Dell'Elice A *et al.* (2021). Filling the gap: A thorough investigation for the genetic diagnosis of unsolved polyposis patients with monoallelic MUTYH pathogenic variants. *Mol Genet Genomic Med*. 9:e1831.
- Fujita M *et al.* (2022). Population-based Screening for Hereditary Colorectal Cancer Variants in Japan. *Clin Gastroenterol Hepatol*, 20(9), 2132-2141.
- Kadiyska T *et al.* (2019). Interstitial Deletion of 5q22.2q23.1 Including APC and TSSK1B in a Patient with Adenomatous Polyposis and Asthenoteratozoospermia. *Mol Syndromol*. 9:235-240.
- Kalbfleisch T *et al.* (2015). Characterization of an APC Promoter 1B deletion in a Patient Diagnosed with Familial Adenomatous Polyposis via Whole Genome Shotgun Sequencing. *F1000Res*. 4:170.
- Khan N *et al.* (2017). Novel mutations and phenotypic associations identified through APC, MUTYH, NTHL1, POLD1, POLE gene analysis in Indian Familial Adenomatous Polyposis cohort. *Sci Rep*. 7:2214.
- Loginova AN et al. (2023). Large Rearrangements in Genes Responsible for Familial Adenomatous Polyposis, MUTYH-Associated Polyposis and Peutz–Jeghers Syndrome in Russian Patients. *Russ J Gastroenterol Hepatol Coloproctol*. Vol. 33, no. 1 pp. 59 – 67.
- Marabelli M *et al.* (2017). A novel APC promoter 1B deletion shows a founder effect in Italian patients with classical familial adenomatous polyposis phenotype. *Genes Chromosomes Cancer*. 56:846-854.
- Naoi D *et al.* (2021). Solid-pseudopapillary neoplasm of the pancreas in a patient with familial adenomatous polyposis: a case report. *Surg Case Rep.* 7:35.
- Nguyen BH at al. (2022). The mutation Spectrum and two novel point mutations in the APC gene in Vietnamese patients with familial adenomatous polyposis. *Asian Pac J Cancer*, 23(5), 1517-1522.
- Papp J *et al.* (2016). Contribution of APC and MUTYH mutations to familial adenomatous polyposis susceptibility in Hungary. *Fam Cancer*. 15:85-97.
- Plawski A *et al.* (2008). APC gene mutations causing familial adenomatous polyposis in Polish patients. *J Appl Genet*. 49:407-414.
- Quadri M *et al.* (2015). APC rearrangements in familial adenomatous polyposis: heterogeneity of deletion lengths and breakpoint sequences underlies similar phenotypes. *Fam Cancer*. 14:41-49.
- Schafer M *et al.* (2016). Neonatal Gardner Fibroma Leads to Detection of Familial Adenomatous Polyposis: Two Case Reports. *European J Pediatr Surg Rep*. 4:17-21.
- Shimamoto Y *et al.* (2021). Gastric neoplasms in patients with familial adenomatous polyposis: endoscopic and clinicopathologic features. *Gastrointest Endosc*. 94:1030-1042 e1032.
- Snow AK *et al.* (2015). APC promoter 1B deletion in seven American families with familial adenomatous polyposis. *Clin Genet*. 88:360-365.
- Svensson S et al. (2022). Merged testing for colorectal cancer syndromes and re-evaluation of genetic variants improve diagnostic yield: Results from a nationwide prospective cohort. *Genes Chromosomes Cancer*, 61(10), 585-591.

- Tanaka K et al. (2022). Small intestinal involvement and genotype-phenotype correlation in familial adenomatous polyposis. *Tech Innov Gastrointest Endosc*, 24(1), 26-34.
- Wang D *et al*. (2019). Adenomatous Polyposis Coli Gene Mutations in 22 Chinese Pedigrees with Familial Adenomatous Polyposis. *Med Sci Monit*. 25:3796-3803.
- Yang M *et al*. (2021). A truncated protein product of the germline variant of the DUOX2 gene leads to adenomatous polyposis. *Cancer Biol Med.* 18:215-226.
- Yanus GA *et al.* (2018). Spectrum of APC and MUTYH germ-line mutations in Russian patients with colorectal malignancies. *Clin Genet*. 93:1015-1021.
- Zhou J *et al.* (2021). A novel large deletion in the APC gene associated with Gardner syndrome in a Chinese family. *Rev Esp Enferm Dig*. 113:179-182.

P043 product history	
Version	*Modification*
E1	Four new *MUTYH* probes and two *GREM1* upstream probes included. One *MUTYH* probe and three reference probes removed.
D1	Six reference probes replaced. Four extra *APC* probes have been included.
C1	Four new *APC* and three new *MUTYH* probes included. Five *APC* probes have been removed. The 88 + 96 nt control fragments replaced (QDX2).
B1	Four *APC* probes have been removed and seven new *APC* probes have been included. Control fragments at 88-96-100-105 nt have been added.
A2	Three extra *APC* probes and two extra reference probes included.
A1	First release.

Implemented changes in the product description

Version E1-08 – 21 March 2024 *(04P)*
- A remark is added on the influence of the SNP rs116020626 on probe signal under tables 1 and 2.
- Exon numbering for the *APC* gene from the MANE Select transcript NM_000038.6 is added in brackets to tables 1 and 2a.
- Various minor textual changes.
- New publications using SALSA MLPA Probemix P043 APC were added to the list.
- Product registered for IVD use in Costa Rica.

Version E1-07 – 24 July 2023 (04P)
- Product is no longer registered as IVD in Morocco.

Version E1-06 – 06 October 2022 (04P)
- Sections Clinical background and Performance characteristics were updated according to recent literature.
- New positive sample for the *GREM1* region included.
- Probe 03324-L29817 (419 nt) is now stated to detect the WT-sequence at the location of c.3183_3187delACAAA mutation instead of the c.3182A>G mutation, because c.3183_3187delACAAA is much more frequent.
- Information regarding the WT-specific *APC* probes, and the associated mutations, has been corrected and updated.
- References and Selected Publications were curated and new literature was included.
- Minor textual and lay-out changes throughout document.

Version E1-05 – 22 July 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended use has become Intended purpose and was rewritten using new template.
- Sections Clinical background and Transcript variants are rewritten for clarification and to include recent knowledge.
- Ligation sites of the probes targeting the *MUTYH* and *GREM1* genes, and the two alternative *APC* transcripts were updated according to new versions of the NM_ reference sequences.
- Recommended positive samples have been updated and are now listed in a table.
- Information was included regarding *APC* Promoter regions 1A and 1B in section about transcript variants and in a note under Table 1 and 2.

- A remark was included in Table 1 and 2 for the variability of the 274 nt probe (15276-L19326).
- Related SALSA MLPA Probemixes was updated.
- References and Selected Publications were curated and new literature was included.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version E1-04 — 26 August 2020 (02P)

- Separate columns for *MUTYH* and *GREM1* were created in Table 1.
- The ligation site of the 11988-L29807 probe (154 nt) in table 2a has been corrected.
- Minor corrections throughout document.

Version E1-03 — 10 October 2019 (02P)

- Product description restructured and adapted to a new template.
- Information about the mutation-specific probes has been clarified.
- Minor textual changes were implemented.
- Intended use and clinical background has been updated.
- Updated the section on transcript variants.
- Table 1 and 2 were updated.
- Table with colorectal cancer probemixes has been included.
- Recent selected publications were included.

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