Product Description
SALSA® MLPA® Probemix P056-D1 TP53

To be used with the MLPA General Protocol.

Version D1.
For complete product history see page 11.

Catalogue numbers:
- **P056-025R:** SALSA MLPA Probemix P056 TP53, 25 reactions.
- **P056-050R:** SALSA MLPA Probemix P056 TP53, 50 reactions.
- **P056-100R:** SALSA MLPA Probemix P056 TP53, 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 P056 TP53 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in *TP53* gene in genomic DNA isolated from human peripheral whole blood specimens. P056 TP53 is intended to confirm a potential cause for and clinical diagnosis of Li-Fraumeni syndrome (LFS1) or Li-Fraumeni-like syndrome (LFL) and for molecular genetic testing of at-risk family members. In addition, this assay can be used to detect deletions or duplications in the human *CHEK2* gene exons 8, 10 and 13 and the 1100delC mutation, to determine a suggested cause for Li-Fraumeni syndrome (LFS2) in a research setting.

Copy number variations (CNVs) detected with P056 TP53 should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *TP53* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

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. In a research setting this assay can be used on tumour tissue-derived DNA.

[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, SALSA Binning DNA SD067 and Coffalyser.Net analysis software.

Clinical background

Li-Fraumeni syndrome (LFS or LFS1) is a clinically and genetically heterogeneous inherited cancer syndrome. LFS is characterised by autosomal dominant inheritance and early onset of tumours, multiple tumours within an individual, and multiple affected family members. The most common types of tumours are adrenocortical carcinomas, breast cancer, central nervous system tumours, osteosarcomas, and soft-tissue sarcomas. Approximately 92% of LFS cases contain germline mutations in the tumour suppressor gene *TP53*. Families presenting incomplete features of LFS are referred to as having Li-Fraumeni-like syndrome (LFL), and 20-40% of these patients have a germline mutation in *TP53* (Ruijs et al. 2010). Around 50% of the individuals carrying germline mutations in *TP53* will develop cancer by the age of 30 years, with a lifetime cancer risk of ≥70% in men and almost ≥90% in women. Individuals with a germline *TP53* pathogenic variant are managed by increased surveillance and are eligible for prophylactic surgery. Next to germline mutations, somatic pathogenic variants in *TP53* are found in about 50% of all tumours, making it one of the most frequently altered genes in human cancers. For more information: https://www.ncbi.nlm.nih.gov/books/NBK1311/.

A second form of LFS (LFS2) has been suggested to be caused by mutations in the *CHEK2* gene, in particular the *CHEK2* 1100delC variant. However, it is currently still under debate whether *CHEK2* gene mutations actually cause LFS or are merely associated with an increased risk of several types of cancer, including those cancers often seen in LFS. Importantly, pathogenic *CHEK2* variants still pose an increased risk of developing cancer, even if *CHEK2* might not be associated with LFS. For more information: https://omim.org/entry/609265.

Gene structure

The *TP53* gene spans ~19 kilobases (kb) on chromosome 17p13.1 and contains 11 exons in the majority of transcripts. The *TP53* LRG_321 is available at www.lrg-sequence.org and is identical to GenBank NG_017013.2.

The *CHEK2* gene is located on chromosome 22q12.1 and spans ~54 kb. The *CHEK2* LRG_302 is identical to GenBank NG_008150.2.

Transcript variants

For *TP53,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform a corresponding to the canonical p53, also known as p53alpha (NM_000546.6; 2512 nt; coding sequence 143-1324). Transcript variant 3 (NM_001126114.3; 2645 nt; coding sequence 143-1168) encodes isoform b (also known as p53beta) and has an additional exon in the 3' coding region, resulting in a shorter and distinct C-terminus compared to isoform a.

For *CHEK2,* multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform a (NM_007194.4; 1844 nt; coding sequence 59-1690).

Exon numbering

The *TP53* exon numbering used in this P056-D1 TP53 product description is the exon numbering from the LRG_321. For *CHEK2* the exon numbering used is from LRG_302. 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 P056-D1 TP53 contains 40 MLPA probes with amplification products between 129 and 490 nucleotides (nt). This includes 15 probes for the *TP53* gene with at least one probe for each exon, and seven flanking probes to determine the extent of the deletion/duplication. Furthermore, there are four probes present for *CHEK2* including a mutation-specific probe for the 1100delC mutation, which will only generate a signal when the mutation is present. In addition, 14 reference probes are included that detect autosomal chromosomal locations. Probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and 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). More information on the use of MLPA in tumour applications can be found in (Homig-Holzel and Savola 2012).

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 reference 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

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 LFS or LFL. 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 number ACC-203 (SK-N-MC cell line) from the Leibniz Institute DSMZ as well as sample ID numbers NA07106 and HG00187 from Coriell Institute have been tested with this P056-D1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change, therefore samples should be validated before use.

Sample ID	Source	Altered target genes in P056-D1	Expected copy number alteration/mutation
ACC-203 *	DSMZ	*TP53* and flanking genes Two reference probe targets	- Heterozygous deletion of the whole *TP53* gene and flanking probes - For *TP53* exons 2a and 2d, the deletion is homozygous Reference probe targets: - Heterozygous duplication of *RAB7A* on 3q - Heterozygous deletion of *UPF2* on 10p

NA07106	Coriell Institute	*CHEK2*	Heterozygous duplication of the *CHEK2* gene
HG00187	Coriell Institute	*CHEK2*	1100delC mutation

* As this is a tumour cell line, reference probes are more prone to show deviating copy number results. Turning off the slope correction in Coffalyser.Net analysis can help to obtain the correct interpretation when regions targeted by reference probes are affected by their copy number.

SALSA Binning DNA SD067

The SD067 Binning DNA provided with this probemix can be used for binning of all probes including the *CHEK2* 1100delC mutation-specific probe (208 nt probe 18318-L26751). SD067 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD067 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 signal. 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 SD067 Binning DNA product description, available online: www.mrcholland.com.

Performance characteristics

Deletions or duplications in *TP53* have been reported in approximately 1% of LFS patients. The remaining *TP53*-related cases are caused by pathogenic *TP53* sequence variants (91%) (Guha and Malkin 2017, Mouchawar et al. 2010). The frequency of *TP53* deletions or duplications in LFL is currently unknown.

Deletions or duplications in *CHEK2* are rare, while the overall prevalence of the *CHEK2* 1100delC mutation in breast cancer is ~0.9%, depending on ethnicity (Zhang et al. 2008). Since the association between *CHEK2* and LFS is under debate, the CHEK2 probes are included in this P056 TP53 probemix for research use only. The analytical sensitivity and specificity for the detection of deletions or duplications in these genes is very high and can be considered >99% (based on a literature review using articles from 2009-2022). 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 *TP53* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), 0 (homozygous deletion) or 4 (homozygous duplication).

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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic/subclonal 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.
- In samples from tumour tissues (research use only), reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

P056 specific notes:

- To determine increased cancer susceptibility caused by *CHEK2* deletions or duplications, the SALSA MLPA Probemix P190 CHEK2 provides a better coverage of the *CHEK2* gene and is recommended.

- *CHEK2* 1100delC probe: we have received reports of experiments in which a peak for the *CHEK2* 1100delC probe appeared in all samples, which was caused by incomplete ligase inactivation. For more information on this issue, please contact info@mrcholland.com. Please note, that this probe will also generate a signal in the unlikely situation that the mutation is present in the *CHEK2* pseudogene. Results obtained with this *CHEK2* mutation probe should therefore be treated with caution.
- The ligation sites of the *TP53* probes 02376-L30912 (exon 4b) at 256 nt and 01999-L21411 (exon 7) at 286 nt are located in a germline and somatic mutational hotspot (https://tp53.isb-cgc.org/). In case of an apparent deletion, the sequence of this region should be sequenced.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *TP53* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P056 TP53.
- 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.
- When used on tumour DNA (for research use only): MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism 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.

As the CHEK2 1100delC mutation-specific probe is only intended to determine the presence of the mutation, positive results obtained for this probe need to be confirmed by sequence analysis to determine the zygosity of the mutation.

Mutation databases

TP53: https://tp53.isb-cgc.org/ and https://databases.lovd.nl/shared/genes/TP53.
CHEK2: https://databases.lovd.nl/shared/genes/CHEK2.
We strongly encourage users to deposit positive results in the corresponding database(s). 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 *TP53* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P056-D1 TP53

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*TP53*	*CHEK2*
64-105	Control fragments – see table in probemix content section for more information			
129	Reference probe 19616-L26684	4p13		
135	Reference probe 16316-L21434	3q21		
140 ¬	EFNB3 probe 03962-L21069		17p13.1	
147 ¬	MPDU1 probe 19643-L26685		17p13.1	
154 * »	**CHEK2 probe** 21913-L06190			**Exon 10**
160	Reference probe 09787-L10202	15q21		
166	**TP53 probe** 01588-L06028		**Exon 1**	
175	**TP53 probe** 19637-L26296		**Downstream**	
181 * ∫ » «	**CHEK2 probe** 21654-L30911			**Exon 13**
187 ¥ ¬	POLR2A probe 19647-L30910		17p13.1	
193	Reference probe 11556-L26606	5q31		
199	**TP53 probe** 01996-L26321		**Exon 2a**	
208 » §	**CHEK2 probe** 18318-L26751			**1100delC mutation**
216	**TP53 probe** 02375-L26750		**Exon 2d**	
224 +	**TP53 probe** 19638-L26297		**Exon 9a**	
230	Reference probe 17130-L26574	11p11		
238 ¬	AKAP10 probe 19648-L00940		17p11.2	
247	Reference probe 08728-L08739	9q21		
256 ¥ ±	**TP53 probe** 02376-L30912		**Exon 4b**	
274 ¥	Reference probe 17450-L30913	16p13		
286 ¥ ±	**TP53 probe** 01999-L21411		**Exon 7**	
299	**TP53 probe** 17420-L21142		**Exon 3**	
310	Reference probe 07028-L06639	14q11		
318	**TP53 probe** 17421-L21315		**Exon 5**	
328	Reference probe 13397-L26608	6q12		
335 ¬	ATP1B2 probe 19884-L26749		17p13.1	
346	**TP53 probe** 17422-L21144		**Exon 10**	
359 ¥	**TP53 probe** 22010-L21147		**Exon 4b**	
372	Reference probe 14835-L26609	1p34		
382 ¬	ATP1B2 probe 19645-L26316		17p13.1	
391	**TP53 probe** 17423-L21145		**Exon 8**	
401	**TP53 probe** 19650-L21141		**Exon 6**	
409	**TP53 probe** 02263-L01749		**Exon 1**	
420	Reference probe 08839-L08899	2p13		
432 ¥ »	**CHEK2 probe** 06631-L30915			**Exon 8**
447 ¥	**TP53 probe** 17424-L30914		**Exon 11**	
459 ¥ ¬	POLR2A probe 09951-L30916		17p13.1	
471	Reference probe 00979-L21316	10p14		
480 ¥	Reference probe 21882-L15817	2q13		
490	Reference probe 19137-L25693	21q22		

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

* New in version D1.
¥ Changed in version D1. Minor alteration, no change in sequence detected.
§ Mutation-specific probe. This probe will generate a signal when the *CHEK2* 1100delC mutation is present. It has been tested on artificial DNA and on positive human samples. However, the probe can give an extra signal due to a simultaneous activity of the ligase and polymerase enzymes.
± Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the NCI *TP53* Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
∫ SNP rs564605612 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 one of the CHEK2 probes in SALSA MLPA Probemix P190.
¬ 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.
+ Exon numbering for Exon 9a is indicated according to LRG_321 transcript t3 (NM_001126114.3). Exon 9a is present in some alternative transcript variants only.

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.

Table 2. P056-D1 probes arranged according to chromosomal location

Table 2a. *TP53*

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location/ Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Centromeric flanking probes					
238 ¬	19648-L00940	*AKAP10*	17p11.2	AGGACCAAGTCA-TGTTGCAATCAA	12.3 Mb
140 ¬	03962-L21069	*EFNB3*	17p13.1	TCTCCTAATTAT-GAGTTCTACAAG	20.5 kb
TP53 on 17p13.1. Ligation sites are according to NM_000546.6, unless otherwise specified.					
		start codon	*143-145 (Exon 2a)*		
409	02263-L01749	Exon 1	127 nt before Exon 1	CTTCCTCCGGCA-GGCGGATTACTT	0.2 kb
166	01588-L06028	Exon 1	58-59	TCCGGGGACACT-TTGCGTTCGGGC	10.8 kb
199	01996-L26321	Exon 2a	117-118	CTCTTGCAGCAG-CCAGACTGCCTT	0.2 kb
216	02375-L26750	Exon 2d	230-231	TTCCTGAAAACA-ACGTTCTGGTAA	0.3 kb
299	17420-L21142	Exon 3	451-450 reverse	TAGCTGCCCTGG-TAGGTTTTCTGG	0.8 kb
256 ±	02376-L30912	Exon 4b	546-547	CAAGATGTTTTG-CCAACTGGCCAA	0.1 kb
359	22010-L21147	Exon 4b	636-637	CATCTACAAGCA-GTCACAGCACAT	0.2 kb
318	17421-L21315	Exon 5	735-736	TATCCGAGTGGA-AGGAAATTTGCG	0.7 kb
401	19650-L21141	Exon 6	831-832	CTCTGACTGTAC-CACCATCCACTA	0.5 kb
286 ±	01999-L21411	Exon 7	981-982	CTGTCCTGGGAG-AGACCGGCGCAC	0.2 kb
391	17423-L21145	Exon 8	1095-1096	CTCTCCCCAGCC-AAAGAAGAAACC	0.2 kb
224 +	19638-L26297	Exon 9a	NM_001126114.3; 1136-1135 reverse	GCTGGTCTGGTC-CTTTAAAATATA	2.7 kb
346	17422-L21144	Exon 10	1188-1189	TGAGGCCTTGGA-ACTCAAGGATGC	1.0 kb
447	17424-L30914	Exon 11	1300-1301	CTCATGTTCAAG-ACAGAAGGGCCT	3.3 kb
175	19637-L26296	downstream	2110 nt after Exon 11 reverse	TGAAGCCATGAG-GAAATTGGGAGA	9.8 kb
		stop codon	*1322-1324 (Exon 11)*		
Telomeric flanking probes					
382 ¬	19645-L26316	*ATP1B2*	17p13.1	AGAACCACCTTG-TCCTCAATTACA	5.0 kb
335 ¬	19884-L26749	*ATP1B2*	17p13.1	CCGCGCCACCAA-GATGGTCATCCA	65.6 kb
147 ¬	19643-L26685	*MPDU1*	17p13.1	GCTGCCCCAGGT-GTTTAAAATCCT	87.8 kb
459 ¬	09951-L30916	*POLR2A*	17p13.1	CGCCAAGTACAT-CATCCGAGACAA	1.8 kb
187 ¬	19647-L30910	*POLR2A*	17p13.1	GAAGACAATGAA-AGTTTTGCGCTG	

Table 2b. *CHEK2*

Length (nt)	SALSA MLPA probe	*CHEK2* exon[a]	Ligation site NM_007194.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*59-61 (Exon 2)*		
432 »	06631-L30915	Exon 8	923-924	TCATCAAGATTA-AAAACTTTTTTG	6.6 kb
154 »	21913-L06190	Exon 10	1079-1080	ACCTTCATGAAA-ACGGTATTATAC	1.1 kb
208 » §	18318-L26751	**1100delC mutation** in Exon 11	1159-1157 reverse	TGCCCAAAATCA-TAATCTAAAATT	1.8 kb
181 ∫ » « #	21654-L30911	Exon 13	1509-1510	CTTAAGACACCC-GTGGCTTCAGGT	
		stop codon	*1688-1690 (Exon 15)*		

[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.

§ Mutation-specific probe. This probe will generate a signal when the *CHEK2* 1100delC mutation is present. It has been tested on artificial DNA and on positive human samples. However, the probe can give an extra signal due to a simultaneous activity of the ligase and polymerase enzymes.
± Ligation site of this probe is located on a common mutational hotspot both in germline and somatic samples as reported by the *TP53* Database (https://tp53.isb-cgc.org/). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
∫ SNP rs564605612 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 one of the CHEK2 probes in SALSA MLPA Probemix P190.
¬ 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.
+ Exon numbering for Exon 9a is indicated according to LRG_321 transcript t3 (NM_001126114.3). Exon 9a is present in some alternative transcript variants only.
# 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.

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.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
372	14835-L26609	*UROD*	1p34	AAGCACCATGGC-TCAGGCCAAGCG	01-045,252
420	08839-L08899	*DYSF*	2p13	TGCCATGAAGCT-GGTGAAGCCCTT	02-071,767
480	21882-L15817	*EDAR*	2q13	AAAGCCCACCAA-GAGGTATGTGGA	02-108,893
135	16316-L21434	*RAB7A*	3q21	CACAATAGGAGC-TGACTTTCTGAC	03-130,000
129	19616-L26684	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
193	11556-L26606	*MYOT*	5q31	CAGATCTCGGCT-ATACCTCCACCA	05-137,250
328	13397-L26608	*EYS*	6q12	ATGGTAAGATTA-ACTGAACCCTCT	06-064,574
247	08728-L08739	*PCSK5*	9q21	GACTATGAAGAA-TGTGTCCCTTGT	09-078,045
471	00979-L21316	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
230	17130-L26574	*MYBPC3*	11p11	GTGCCTCAGTGA-CCAGGCTGGCTC	11-047,310
310	07028-L06639	*RPGRIP1*	14q11	GAGGTTCCCATT-GAAGCTGGCCAG	14-020,866
160	09787-L10202	*SPG11*	15q15	GGGACACATTCA-GGACTCAACAGA	15-042,675
274	17450-L30913	*GRIN2A*	16p13	TGCAGGATTATA-ATCTCACAATCT	16-009,761
490	19137-L25693	*PSMG1*	21q22	TGGAAGCTTTTA-AGCCTATACTTT	21-039,471

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

Related SALSA MLPA probemixes

P190 CHEK2: Contains probes for *CHEK2*, *ATM*, *TP53*, involved in breast cancer.
P002/P087 BRCA1: Contain probes for the *BRCA1* gene, involved in breast and ovarian cancer.
P045/P077/P090 BRCA2: Contain probes for the *BRCA2* gene, involved in breast and ovarian cancer.
P225 PTEN: Contains probes for the *PTEN* gene, involved in Cowden syndrome.

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P056 product history	
Version	*Modification*
D1	Two flanking probes of *TP53* and all flanking probes of *CHEK2* have been removed. One CHEK2 probe has been added and one CHEK2 probe has been replaced. Moreover, nine probes have been changed in length but not in the sequence detected.
C1	Most of the reference and flanking probes are replaced and several are added. Furthermore, one probe for *TP53* exon 9a and one probe downstream *TP53* exon 11 are added. Moreover, one CHEK2 probe is replaced and one added.
B1	All reference probes and several target probes have been replaced and an additional probe for *TP53* exon 5 included. In addition, the 88 and 96 nt control fragments have been replaced.
A2	One reference probe has been replaced, five probes have a small change in length (but no change in sequence detected) and four extra control fragments at 88-96-100-105 nt have been included.
A1	Several new 17p probes have been added as compared to previous lots.
A	First release.

Implemented changes in the product description

Version D1-05– 22 July 2022 (04P)
- Clinical background, Performance characteristics sections and P056 specific notes updated to contain additional information about *CHEK2* in relation to LFS/cancer predisposition.
- Confirmation of *CHEK2* 1100delC mutation added to Confirmation of results section.
- Positive control samples NA07106 and HG00187 added to the Positive control DNA samples section.
- Links to mutation databases updated.
- Probe warnings for the CHEK2 1100delC-specific probe in Table 1 and 2 rephrased.
- SNP warning added for the 181 nt probe in Table 1 and 2.
- Multiple minor textual and layout changes.

Version D1-04– 18 March 2021(04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- Performance characteristics section updated.
- Ligation sites of the probes targeting the *TP53* gene updated according to new version of the NM_000546.6 reference sequence.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Added two new references and removed one in 'Selected publications using SALSA MLPA Probemix P056 TP53' section on page 10.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version D1-03 – 29 April 2020 (04)
- Product is now registered for IVD use in Israel.

Version D1-02 – 05 October 2018 (04)

- Ligation sites of the probes targeting the *CHEK2* gene updated according to new version of the NM_reference sequence.
- Note was added under Table 1 and 2 for CHEK2 probes that have the same sequence as probes in the probemix P190.

Version D1-01 – 27 June 2018 (04)
- 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).
- New references added.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- SD binning number changed from SD029 to SD067.
- Warning for SNPs 216nt and 256nt probes removed under Table 1 and 2.

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