Instructions for Use
SALSA® MLPA® Probemix P056 TP53

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit and SALSA® Binning DNA SD067, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P056 TP53 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P056 TP53**
Version	**D1**
Catalogue numbers	**P056-025R (25 reactions)** **P056-050R (50 reactions)** **P056-100R (100 reactions)**
Basic UDI-DI	**872021148P0565X**
Ingredients	**Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA**

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL COSTA RICA
RUO	ALL OTHER COUNTRIES

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD067	SD067

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in this Product Version

As compared to C1 version

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.

1. Intended Purpose

The SALSA MLPA Probemix P056 TP53 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *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 (LFS) 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 c.1100delC mutation, for differential diagnosis of predisposition to *CHEK2*-related cancer types in patients that were initially suspected of LFS/LFL.

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.

Please note that this probemix covers all exons of *TP53* but not of *CHEK2*. For *CHEK2*, SALSA MLPA Probemix P190 CHEK2 provides a better coverage and may detect aberrations that are not detected by this P056 TP53 probemix.

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 IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2]To be used in combination with a SALSA MLPA Reagent Kit, SALSA Binning DNA SD067 and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • salting out (manual)

Sample Types		
Test Sample	• provided by user	
Reference Samples (Required)	• provided by user • extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • have a normal copy number and ≤0.10 standard deviation for all probes except for the mutation-specific probe. • at least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of LFS or LFL.	
No-DNA Control (Preferably)	• provided by user • $TE_{0.1}$ buffer instead of DNA • to check for DNA contamination	
Binning DNA (Initial Experiment)	• SALSA Binning DNA SD067, provided by MRC Holland • required in initial experiment to determine suitable bin set • should never be used as a reference sample	
Positive Control Samples (Preferably)	• provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• In the validation experiments of this probemix, the peaks of the mutation-specific probes are expected to be absent in the majority of samples from healthy individuals.	

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of Mutation-Specific Probes

Signal Strength	Mutation Status
≥10% median peak height reference probes	Mutation *CHEK2* c.1100delC detected (expected only in positive samples)
<10% median peak height reference probes	Mutation *CHEK2* c.1100delC not detected (expected in most samples from healthy individuals)

These final ratios are only valid for germline testing.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy numbers in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table above can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P056 TP53 in 41 samples from healthy individuals with normal copy number and seven samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.</td></tr>
<tr><td>Expected values for point mutation detection in normal and affected populations</td><td>The mutation-specific probe will only generate a signal when the CHEK2 c.1100delC mutation is present. Please note that background signals of the mutation-specific probe can be expected above the threshold in some cases. Users should always compare the relative peak height of the mutation-specific probe in mutation-positive samples to the relative peak height in reference samples. A clear signal (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes.
The expected value for the mutation-specific probe was verified with SALSA MLPA Probemix P056 TP53 using one mutation-positive sample, seven samples positive for other aberrations detected by P056 TP53, and 41 samples from healthy individuals without the CHEK2 c.1100delC mutation, and the expected results were found in all tested samples.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P056 TP53 on four samples with known CNVs/mutation status and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using SALSA MLPA Probemix P056 TP53 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/mutation status. For most probes, expected FRs/signals (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 198/204 measurements
Mutation: Expected signal for 12/12 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 204/204 measurements
Mutation: Expected signal for 12/12 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 203/204 measurements
Mutation: Expected signal for 12/12 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 204/204 measurements
Mutation: Expected signal for 12/12 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 149/204 measurements
Mutation: Expected signal for 12/12 measurements</td></tr>
</table>
* Results are summarised for all probes across all four samples tested in triplicate.

NaCl, $FeCl_3$ and heparin did not interfere with copy number determination, while an effect on the FRs was observed for a low number of probes with EDTA. Hemoglobin had the largest effect on the FRs; ambiguous as well as false results were obtained. None of the interferents had an effect on the determination of mutation status.

To minimise variability across samples, all samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible.</td></tr>
</table>

Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, four previously genotyped samples were tested using SALSA MLPA Probemix P056 TP53 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutations status over multiple days, and by multiple operators. Results showed a correct call in 970/972 data points, leading to a precision of >99%.
Clinical validity*	*TP53*: Deletions/duplications in the *TP53* gene are detected in ~1% of LFS/LFL patients. *CHEK2*: Deletions/duplications in the *CHEK2* gene are detected by P056 TP53 in <1% of individuals with a predisposition to *CHEK2*-related cancer. The *CHEK2* c.1100delC mutation is the most common founder mutation of this gene and it is of Northern and Eastern European origin. Therefore, the frequency of *CHEK2* c.1100delC mutation varies across populations. *Based on a 2009-2024 literature review

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Mutation	Length (nt)	Probe number	Warnings
17p13.1	*POLR2A*	Exon 3	1.8 kb		187	19647-L30910	¬
17p13.1	*POLR2A*	Exon 8	87.8 kb		459	09951-L30916	¬
17p13.1	*MPDU1*		65.6 kb		147	19643-L26685	¬
17p13.1	*ATP1B2*	Exon 1	5.0 kb		335	19884-L26749	¬
17p13.1	*ATP1B2*	Exon 7	9.8 kb		382	19645-L26316	¬
17p13.1	*TP53*	Downstream	3.3 kb		175	19637-L26296	Ø
17p13.1	*TP53*	Exon 11	1.0 kb		447	17424-L30914	
17p13.1	*TP53*	Exon 10	2.7 kb		346	17422-L21144	
17p13.1	*TP53*	Intron 9 (Exon 9a)	0.2 kb		224	19638-L26297	Ø
17p13.1	*TP53*	Exon 9 (8)	0.2 kb		391	17423-L21145	
17p13.1	*TP53*	Exon 8 (7)	0.5 kb		286	01999-L21411	∫
17p13.1	*TP53*	Exon 7 (6)	0.7 kb		401	19650-L21141	
17p13.1	*TP53*	Exon 6 (5)	0.2 kb		318	17421-L21315	
17p13.1	*TP53*	Exon 5 (4b)	0.1 kb		359	22010-L21147	
17p13.1	*TP53*	Exon 5 (4b)	0.8 kb		256	02376-L30912	∫
17p13.1	*TP53*	Exon 4 (3)	0.3 kb		299	17420-L21142	
17p13.1	*TP53*	Exon 3 (2d)	0.2 kb		216	02375-L26750	
17p13.1	*TP53*	Exon 2 (2a)	10.8 kb		199	01996-L26321	
17p13.1	*TP53*	Exon 1	0.2 kb		166	01588-L06028	
17p13.1	*TP53*	Upstream (Exon 1)	20.5 kb		409	02263-L01749	Ø
17p13.1	*EFNB3*		12.3 **M**b		140	03962-L21069	¬
17p11.2	*AKAP10*				238	19648-L00940	¬
22q12.1	*CHEK2*	Exon 13	1.8 kb		181	21654-L30911	# « »
22q12.1	*CHEK2*	Exon 11	1.1 kb	c.1100delC	208	18318-L26751	§ »
22q12.1	*CHEK2*	Exon 10	6.6 kb		154	21913-L06190	« »
22q12.1	*CHEK2*	Exon 8			432	06631-L30915	« »
1p	Reference				372	14835-L26609	
2p	Reference				420	08839-L08899	
2q	Reference				480	21882-L15817	
3q	Reference				135	16316-L21434	
4p	Reference				129	19616-L26684	
5q	Reference				193	11556-L26606	
6q	Reference				328	13397-L26608	
9q	Reference				247	08728-L08739	
10p	Reference				471	00979-L21316	
11p	Reference				230	17130-L26574	
14q	Reference				310	07028-L06639	
15q	Reference				160	09787-L10202	
16p	Reference				274	17450-L30913	
21q	Reference				490	19137-L25693	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *TP53* and *CHEK2* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of one probe with a target at the edge of or slightly outside the coding region is altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18

7. Precautions and Warnings

Probe warnings

§ This probe will only generate a signal when the *CHEK2* c.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.

¬ This probe is a flanking probe, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.

« This probe is 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.

The specificity of this probe 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.

» Detects the same sequence as the *CHEK2* probe in SALSA® MLPA® Probemix P190 CHEK2.

∫ 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.

Ø This probe targets a sequence outside of the known coding region of the MANE Select transcript.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard

Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).

2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. *CHEK2* c.1100delC mutation-specific probe: We have received reports of experiments in which a peak for the *CHEK2* c.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* c.1100delC probe should therefore be treated with caution.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The *CHEK2* c.1100delC mutation-specific probe is only intended to determine the presence (or absence) of the mutation.

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description
Version D1-06 – 21 March 2025 (03S) - Updated to a new template. - Intended purpose was updated, specifying the testing population and clinical application of *CHEK2* copy number determination, updating disease nomenclature, and specifying assay is manual. - Exon numbering updated for *TP53* probes. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Warning about low signal caused by salt contamination added to 154 nt and 432 nt probes. - Warning for extra signal due to incomplete ligase inactivation added to 208 nt probe. - SNV rs564605612 can affect the probe signal. However, the warning for this SNV present in previous product description versions have been replaced by a general warning for small sequence changes, included in the section Precautions and Warnings. - Probemix is now registered for IVD use in Costa Rica. - Probemix is now IVDR certified.