Instructions for Use
SALSA® MLPA® Probemix P190 CHEK2

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit and SALSA® Binning DNA SD078, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P190 CHEK2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P190 CHEK2**
Version	**D1**
Catalogue numbers	**P190-025R (25 reactions)** **P190-050R (50 reactions)** **P190-100R (100 reactions)**
Basic UDI-DI	**872021148P19064**
Ingredients	**Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA**

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD078	SD078

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.

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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

Compared to C1 version

16 target probes have been added and several target probes have been adjusted, replaced or removed. Almost all reference probes have been replaced and two were added. Several probes have a small change in length but no change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P190 CHEK2 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 human *CHEK2*, and the presence of the *CHEK2* c.1100delC mutation in genomic DNA isolated from human peripheral whole blood specimens. P190 CHEK2 is intended to confirm a potential cause for and clinical diagnosis of a predisposition to breast cancer and other *CHEK2*-related cancer types. In addition, deletions and duplications in the human *ATM* and *TP53* genes can be detected with this probemix for differential diagnosis of *ATM*- or *TP53*-related predisposition to cancer, respectively, in patients originally suspected of a *CHEK2*-related cancer predisposition. This product can also be used to determine increased cancer susceptibility in at-risk family members.

Copy number variations (CNVs) detected with P190 CHEK2 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 *CHEK2*, *ATM* and *TP53* genes are point mutations, of which only the *CHEK2* c.1100delC mutation 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 *CHEK2* but not of *ATM* and *TP53*. For the latter two genes, the SALSA MLPA Probemixes P041/P042 ATM and SALSA MLPA Probemix P056 TP53 provide a better coverage and may detect aberrations that are not detected by this P190 CHEK2 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, e.g. from DNA extracted from formalin-fixed paraffin embedded (FFPE) or fresh tumour materials.

[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 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 probes. • at least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of cancer predisposition.	
No-DNA Control (Preferably)	• provided by user • $TE_{0.1}$ buffer instead of DNA • to check for DNA contamination	
Binning Sample (Initial Experiment)	• SALSA Binning DNA SD078, 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 probe 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)

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 P190 CHEK2 in 42 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 values for the mutation-specific probe were verified with SALSA MLPA Probemix P190 CHEK2 using one mutation positive sample, six samples positive for other aberrations detected by P190 CHEK2, and 42 samples from healthy individuals without the CHEK2 c.1100delC mutation, and the expected signal percentage was 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 P190 CHEK2 on five samples with known CNVs/mutations and on one sample without any mutation. Expected results were obtained in all samples 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 P190 CHEK2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on six samples with known CNVs/mutations 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 730/738 measurements
Mutation: Expected signal for 18/18 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 731/738 measurements
Mutation: Expected signal for 18/18 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 736/738 measurements
Mutation: Expected signal for 18/18 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 735/738 measurements
Mutation: Expected signal for 18/18 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 661/738 measurements
Mutation: Expected signal for 18/18 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. Seven probes for *CHEK2* target sequences for which homologous regions were identified. 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, eight previously genotyped samples were tested using SALSA MLPA Probemix P190 CHEK2 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutations over multiple days, and by multiple operators. Results showed a correct call in 100% between triplicates for both copy number determination and determination of mutation status. Reproducibility between operators and days is 98.6% for copy number determination and 100% for determination of the mutation status. Overall, precision is >99%.
Clinical validity*	*CHEK2*: Deletions/duplications in the *CHEK2* gene are detected in 0-3.5% 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. *ATM*: Deletions/duplications in the *ATM* gene are detected in <1% of individuals with a predisposition to *ATM*-related cancer. *TP53*: Deletions/duplications in *TP53* gene are detected in <1% of individuals with a predisposition to *TP53*-related cancer. *Based on a 2008-2024 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
11q22.3	*ATM*	Exon 1	12.8 kb		328	21665-L30287	+
11q22.3	*ATM*	Exon 5	11.2 kb		436	21668-L30290	
11q22.3	*ATM*	Exon 8	2.1 kb		292	21663-L30525	
11q22.3	*ATM*	Exon 9	8.5 kb		238	21660-L30282	
11q22.3	*ATM*	Exon 15	9.8 kb		483	21672-L30294	
11q22.3	*ATM*	Exon 17	4.0 kb		286	21662-L30284	
11q22.3	*ATM*	Exon 20	9.8 kb		232	21659-L30281	
11q22.3	*ATM*	Exon 24	8.5 kb		196	21657-L30279	
11q22.3	*ATM*	Exon 29	7.7 kb		474	21671-L30293	
11q22.3	*ATM*	Exon 33	10.6 kb		319	21664-L30286	
11q22.3	*ATM*	Exon 38	9.5 kb		160	21652-L30274	
11q22.3	*ATM*	Exon 43	3.8 kb		136	21651-L30273	
11q22.3	*ATM*	Exon 45	7.7 kb		445	21669-L30291	
11q22.3	*ATM*	Exon 49	6.0 kb		263	21661-L30283	
11q22.3	*ATM*	Exon 55	10.8 kb		184	21655-L30277	
11q22.3	*ATM*	Exon 58	9.0 kb		131	21650-L30272	
11q22.3	*ATM*	Exon 61	10.3 kb		202	21658-L30280	
11q22.3	*ATM*	Exon 62	0.3 kb		463	21670-L30292	
11q22.3	*ATM*	Exon 63			190	21656-L30840	
17p13.1	*TP53*	Exon 11	3.9 kb		346	00345-L31314	
17p13.1	*TP53*	Exon 9 (8)	2.7 kb		166	21653-L30275	
17p13.1	*TP53*	Exon 4 (3)	11.2 kb		226	01997-L31312	
17p13.1	*TP53*	Exon 1			281	21581-L25982	
22q12.1	*CHEK2*	Exon 15	1.0 kb		256	07281-SP0890-L30457	« Ж #
22q12.1	*CHEK2*	Intron 14 (Exon 14)	0.4 kb		244	06636-L30524	« # Ø
22q12.1	*CHEK2*	Exon 14	4.7 kb		382	21666-L30288	« # +
22q12.1	*CHEK2*	Exon 13	0.1 kb		178	21654-L30276	« # П
22q12.1	*CHEK2*	Exon 13	0.9 kb		355	19654-L26320	« # +
22q12.1	*CHEK2*	Exon 12	0.6 kb		143	21419-L29916	« # +
22q12.1	*CHEK2*	Exon 11	0.2 kb		172	21418-L29915	« # +
22q12.1	*CHEK2*	Exon 11	1.1 kb	c.1100delC	313	22034-SP0468-L31261	§ Ж » П
22q12.1	*CHEK2*	Exon 10	2.9 kb		418	06632-L06190	« П
22q12.1	*CHEK2*	Exon 9	3.6 kb		400	02579-L02041	« ∫ »
22q12.1	*CHEK2*	Exon 8	6.5 kb		427	06631-L06189	« П
22q12.1	*CHEK2*	Exon 7	2.0 kb		154	06630-L07119	
22q12.1	*CHEK2*	Exon 6	7.5 kb		208	06629-L30453	
22q12.1	*CHEK2*	Exon 5	5.6 kb		373	06628-L06186	
22q12.1	*CHEK2*	Exon 4	0.2 kb		274	06627-L06185	
22q12.1	*CHEK2*	Exon 3	9.2 kb		306	06626-L30841	
22q12.1	*CHEK2*	Exon 2	7.3 kb		391	06625-L06183	
22q12.1	*CHEK2*	Exon 1	0.2 kb		337	06624-L24131	»
22q12.1	*CHEK2*	Upstream (Exon 1)	1.9 kb		220	06623-L31306	Ø
22q12.1	*HSCB*				268	06800-L30458	¬
2p	Reference				250	17871-L22467	
2q	Reference				148	14199-L23450	
3p	Reference				298	15388-L17790	
4p	Reference				490	20096-L27538	
5p	Reference				454	18691-L02476	
5q	Reference				124	18709-L21056	
6p	Reference				214	10730-L30523	
9q	Reference				409	08725-L08736	
11p	Reference				364	18676-L24030	
20q	Reference				500	17001-L22947	

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 *CHEK2*, *ATM*, and *TP53* 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 several probes with a target at the edge of or slightly outside the coding region are 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.
Ж	This probe consists of three parts and has 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. When this occurs in reference samples, it can look like an increased signal in the test samples.
#	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* probes in SALSA® MLPA® Probemix P045 BRCA2/CHEK2.
Π	Detects the same sequence as the *CHEK2* probes in SALSA® MLPA® Probemix P056 TP53.
∫	Probe insensitive to depurination. A high signal of the 400 nt probe can be due to depurination of the sample DNA, e.g. due to insufficient buffer concentration in the DNA sample or a prolonged denaturation time. Reduced signals of other probes caused by sample depurination lead to seemingly high signals of the 400 nt probe.
+	The ligation site of this probe is >20 nt away from the nearest exon. For more information, download the probe sequence sheet from the probemix-specific page on www.mrcholland.com.
Ø	This probe targets a sequence outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance.

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. *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. This was due to simultaneous ligase and polymerase activity caused by either incomplete heat inactivation of Ligase-65 or contamination of ligase mastermix with polymerase mastermix or vice versa. For more information on this issue, please contact info@mrcholland.com. Results obtained with this *CHEK2* c.1100delC probe should therefore be treated with caution.
5. Deletions of the last exons of *ATM* (exon 62-63) are encountered relatively frequently (own validation observations, Micol et al. 2011, Nakamura et al. 2012, Podralska et al. 2014, Susswein et al. 2016, Tung et al. 2015). *Duplication* of these exons might not result in inactivation of that gene copy and should therefore be interpreted with caution. In the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), these variants are classified as variant of uncertain significance (VUS)/likely benign.
6. Copy number alterations of reference probes are unlikely to be related to the condition tested.

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.

9. References Cited in this IFU

1. Micol R et al. (2011). Morbidity and mortality from Ataxia-Telangiectasia are associated with ATM genotype. J Allergy Clin Immunol. 128:382-9. E381.
2. Nakamura K et al. (2012). Functional characterization and targeted correction of ATM mutations identified in Japanese patients with Ataxia-Telangiectasia. Hum Mutat. 33:198-208.
3. Podralska MJ et al. (2014). Ten new ATM alterations in Polish patients with Ataxia-Telangiectasia. Mol Genet Genomic Med. 2:504-11.
4. Susswein LR et al. (2016). Pathogenic and likely pathogenic variant prevalence among the first 10,000 patients referred for next-generation cancer panel testing. Genet Med. 18:823-32.
5. Tung N et al. (2015). Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer. 121:25-33.

Implemented changes in the product description
Version D1-07 – 21 March 2025 (03S) - Updated to a new template. - Intended purpose was updated, specifying the testing population and clinical application of *ATM* and *TP53* copy number determination, and specifying assay is manual. - Reference to SALSA Binning DNA SD078 removed from the intended purpose footnote. - 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. - SNV rs564605612 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes in the section Precautions and warnings. - Probemix is now IVDR certified.