Product Description
SALSA® MLPA® Probemix P190-D1 CHEK2

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 13.

Catalogue numbers:
- **P190-025R:** SALSA MLPA Probemix P190 CHEK2, 25 reactions.
- **P190-050R:** SALSA MLPA Probemix P190 CHEK2, 50 reactions.
- **P190-100R:** SALSA MLPA Probemix P190 CHEK2, 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 P190 CHEK2 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 human *CHEK2*, *ATM* and *TP53* genes, and for the detection of the *CHEK2* c.1100delC variant in genomic DNA isolated from human peripheral whole blood specimens. P190 CHEK2 is intended to confirm a potential cause for breast cancer in patients who are negative for *BRCA1*, *BRCA2* and *PALB2* mutations, and for other *CHEK2*-related cancer types, including colorectal cancer. 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 P041/P042 ATM and P056 TP53 probemixes 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 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 SD078.

Clinical background

CHEK2, *ATM* and *TP53* all play important roles in DNA damage repair. A defect in one of these genes can lead to an increased risk of tumour formation. For breast cancer, autosomal dominant mutations in the genes *BRCA1* and *BRCA2* are the most frequent cause, followed by mutations in *CHEK2*, *ATM* and *PALB2*, though with a much lower frequency (Buys et al. 2017). Mutations in *CHEK2* may also increase the risk of developing colorectal cancer (Xiang et al. 2011) and other cancers, including prostate cancer (Cybulski et al. 2006). Mutations in *CHEK2*, and the c.1100delC mutation in particular, have also been suggested as an underlying cause of Li-Fraumeni syndrome (LFS) type 2. Moreover, a deletion in *CHEK2* was found in a patient fulfilling Li-Fraumeni-Like (LFL) criteria (Ruijs et al. 2009). Researchers are uncertain whether *CHEK2* mutations actually cause LFS or are merely associated with an increased risk of several types of cancer, including cancers seen in LFS. For more information: https://omim.org/entry/609265.

CHEK2 exons 11-15 share a high sequence homology with several *CHEK2* pseudogenes, which can result in a pseudogene-mediated gene conversion (Pan et al. 2017).

Autosomal dominant mutations in *ATM* are linked to an increased risk of cancer, with breast cancer in particular (see Table 1). Autosomal recessive mutations of *ATM* cause Ataxia-Telangiectasia, which is characterized by progressive cerebellar ataxia, telangiectases, and a predisposition to malignancy, particularly leukaemia and lymphoma. For more information: https://www.ncbi.nlm.nih.gov/books/NBK26468/.

Autosomal dominant *TP53* mutations result in LFS. The most common types of tumours in LFS are soft tissue sarcomas and osteosarcomas, pre-menopausal breast cancer, brain tumours, leukaemia, and adrenocortical carcinoma. Families presenting incomplete features of LFS are referred to as having LFL, and around 20-40% of these patients have a germline mutation in *TP53* (Ruijs et al. 2010). More information on LFS: https://www.ncbi.nlm.nih.gov/books/NBK1311/.

Table 1. Overview of different types of cancer caused by mutations in *CHEK2, ATM* and *TP53*, and the associated relative risks of developing these cancers.

Gene	Cancer	Relative risk	Occurrence mutations*	References[#]
CHEK2	Breast	Lifetime risk of 25-39% in heterozygotes, dependent on the variant and family history	0-3.5% depending on ethnicity	PMID: 18172190, 21876083, 18381420, 28085182, 15122511, 27595995
	Other cancers, such as colorectal and prostate	Only preliminary evidence	Not known	PMID: 21807500, 25431674, 17085682, 24506336, 22901170
ATM	Breast	Lifetime risk of 17-52%, but can be dependent on the variant	~1%	PMID: 26523341, 28085182 16832357, 19781682, 22585167, 27595995
	Other cancers, such as pancreatic, ovarian and prostate	Only preliminary evidence	Not known	PMID: 22585167, 26483394, 27433846, 18565893, 29348823, 29486991
TP53	(Pre- menopausal) Breast	Lifetime risk of ~79%	<1%	PMID: 20522432, 28085182, 25467110, 26523341
	Other cancers, such as sarcomas and brain tumours	Lifetime risk of cancer in general for men is 73%; for women this is nearly 100%	~80% of families with features of LFS	PMID: 10864200, 20522432

* Including point mutations, indels, deletions and duplications. Percentages depend on the population tested. For example, these percentages may be higher in a *BRCA1/2* mutation negative population.
[#] PMID: PubMed unique identifier.

Gene structure

The *CHEK2* gene spans 54 kilobases (kb) on chromosome 22q12.1 and contains 15 exons. The *CHEK2* LRG_302 is available at www.lrg-sequence.org and is identical to GenBank NG_008150.2.

The *ATM* gene spans 146 kb on chromosome 11q22.3 and contains 63 exons. The *ATM* LRG_135 is available and is identical to GenBank NG_009830.1.

The *TP53* gene spans 19 kb on chromosome 17p13.1 and contains 11 exons in the majority of transcripts. The *TP53* LRG_321 is available and is identical to GenBank NG_017013.2.

Transcript variants

For *CHEK2*, multiple transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/11200). Transcript variant 1 is the most predominant transcript and encodes isoform a (NM_007194.4; 1844 nt; coding sequence 59-1690). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon in exon 15.

For *ATM*, multiple transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/472). Transcript variant 2 is a reference standard in the NCBI RefSeq project (NM_000051.4; 12915 nt; coding sequence 151-9321). The ATG translation start site is located in exon 2 and the stop codon is located in exon 63. *ATM* transcript variant 1 differs from transcript variant 2 in the 5' UTR. Both transcripts encode isoform a.

For *TP53*, multiple transcript variants have been described (https://www.ncbi.nlm.nih.gov/gene/7157). Transcript variant 1 encodes isoform a (also known as p53alpha), which is the longest isoform (NM_000546.6; 2512 nt; coding sequence 143-1324). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2a and the stop codon in exon 11.

Exon numbering

The *CHEK2* exon numbering used in this P190-D1 CHEK2 product description is the exon numbering from the LRG_302 sequence. The *ATM* exon numbering used is from the LRG_135 sequence and the *TP53* exon numbering is from the LRG_321 sequence. 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 P190-D1 CHEK2 contains 53 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 19 probes for the *CHEK2* gene and one probe for the *HSCB* gene, just upstream of *CHEK2*. This probemix also contains one probe specific for the *CHEK2* c.1100delC mutation, which will only generate a signal when the mutation is present. Moreover, 19 probes for *ATM* and four probes for *TP53* are also present. These probes target sequences in various parts of the genes, including in the first and last exon. In addition, ten 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.

Required specimens
Extracted DNA from peripheral whole blood specimens, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of hereditary 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 HG00187, NA07106, HG01872, HG00343, NA08618 and HG03694 from the Coriell Institute have been tested with this P190-D1 probemix at MRC Holland and can be used as positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Corriell Sample ID	Altered target genes in P190-D1	Expected copy number alteration/mutation
HG00187	*CHEK2*	*CHEK2* c.1100delC mutation
NA07106	*CHEK2*	Heterozygous duplication of the complete *CHEK2* gene and the *HSCB* flanking probe
HG01872	*CHEK2*	Heterozygous duplication of *CHEK2* exon 3-5
HG00343	*CHEK2*	Heterozygous deletion of *CHEK2* exon 9-10
NA08618	*ATM*	Heterozygous duplication of the complete *ATM* gene
HG03694	*ATM*	Heterozygous duplication of *ATM* exon 62-63

SALSA Binning DNA SD078
The SD078 Binning DNA provided with this probemix can be used for binning of all probes including one mutation-specific probe (313 nt probe 22034-SP0468-L31261 *CHEK2* c.1100delC mutation). SD078 Binning DNA is a mixture of genomic DNA from healthy individuals and synthetic DNA that contains the target

sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD078 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 SD078 Binning DNA product description, available online: www.mrcholland.com.

Performance characteristics

Deletions or duplications in *CHEK2* are found in <1% of breast cancer patients (Apostolou et al. 2018, Havranek et al. 2015, Ruijs et al. 2009, Tedaldi et al. 2014), although *CHEK2* exon 9-10 deletions are more frequent (~1.0%) in Slavic populations (Cybulski et al. 2007, Walsh et al. 2006). The overall prevalence of the *CHEK2* c.1100delC mutation in breast cancer is ~0.9%, although the mutation has a higher prevalence in patients with a family history of breast cancer or patients from Northern European origin (Zhang et al. 2008).

Ataxia-Telangiectasia, which is characterized by cerebellar ataxia, telangiectases and a predisposition to malignancy, is caused by CNVs of the *ATM* gene in 1-2% of cases. In breast cancer patients, the frequency of *ATM* deletions or duplications is less than 0.1% (Susswein et al. 2016, Tung et al. 2015). Please note that only 19 out of 63 exons are covered in this probemix, which means that not all deletions or duplications can be detected with this probemix.

The frequency of deletions or duplications in *TP53* in Li-Fraumeni syndrome or Li-Fraumeni-like is ~1% in *TP53* point mutation negative patients (https://www.ncbi.nlm.nih.gov/books/NBK1311/, Mouchawar et al. 2010, Schulz et al. 2012). In breast cancer patients without an indication for Li-Fraumeni syndrome, the frequency will be lower (Mouchawar et al. 2010, Susswein et al. 2016). Moreover, only 4 out of 11 exons are covered in this probemix, which means that not all deletions or duplications can be detected.

The percentages mentioned above are dependent on the population tested and are likely to be higher in *BRCA1*/*BRCA2*/*PALB2* mutation negative patients.

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 2008-2022 literature review).

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 *CHEK2*, *ATM* and *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

The above mentioned FR values do not apply to the CHEK2 c.1100delC mutation-specific probe. The peak of the mutation-specific probe is expected to be absent in the majority of samples tested, but ~0.9% of breast cancer patients are carriers for this mutation. The prevalence is higher in patients with a family history of breast cancer or patients from Northern European origin. A clear signal (at least 10% of the median peak height of all reference probes in that sample) for this probe indicates that the mutation is present.

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P190 specific notes:

- CHEK2 c.1100delC mutation-specific probe: We have received reports of experiments in which a peak for the CHEK2 c.1100delC mutation-specific probe appeared in *all* samples, which was caused by incomplete ligase inactivation. For more information on this issue, please contact info@mrcholland.com. Results

obtained with this CHEK2 mutation probe should therefore be treated with caution, in particular when a signal is observed in all samples in an experiment.
- 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.

Limitations of the procedure
- In most populations, the major cause of genetic defects in the *CHEK2, ATM* or *TP53* genes are small (point) mutations, of which only the *CHEK2* c.1100delC mutation will be detected by using SALSA MLPA Probemix P190 CHEK2.
- The CHEK2 c.1100delC mutation-specific probe is only intended to determine the presence (or absence) of the mutation.
- Not all exons of *ATM* and *TP53* are covered. 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 always require confirmation by another method. Because the CHEK2 c.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.

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. The presence of the *CHEK2* c.1100delC mutation should always be confirmed by sequence analysis.

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

Table 2. SALSA MLPA Probemix P190-D1 CHEK2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*CHEK2*	*ATM*	*TP53*
64-105	Control fragments – see table in probemix content section for more information				
124 *	Reference probe 18709-L21056	5q			
131 *	**ATM probe** 21650-L30272			**Exon 58**	
136 *	**ATM probe** 21651-L30273			**Exon 43**	
143 ¥ «	**CHEK2 probe** 21419-L29916		**Exon 12**		
148 *	Reference probe 14199-L23450	2q			
154	**CHEK2 probe** 06630-L07119		**Exon 7**		
160 *	**ATM probe** 21652-L30274			**Exon 38**	
166 *	**TP53 probe** 21653-L30275				**Exon 8**
172 « ¥	**CHEK2 probe** 21418-L29915		**Exon 11**		
178 * « ± Π	**CHEK2 probe** 21654-L30276		**Exon 13**		
184 *	**ATM probe** 21655-L30277			**Exon 55**	
190 *	**ATM probe** 21656-L30840			**Exon 63**	
196 *	**ATM probe** 21657-L30279			**Exon 24**	
202 *	**ATM probe** 21658-L30280			**Exon 61**	
208 ¥	**CHEK2 probe** 06629-L30453		**Exon 6**		
214 *	Reference probe 10730-L30523	6p			
220 ¥	**CHEK2 probe** 06623-L31306		**Exon 1**		
226 ¥	**TP53 probe** 01997-L31312				**Exon 3**
232 *	**ATM probe** 21659-L30281			**Exon 20**	
238 *	**ATM probe** 21660-L30282			**Exon 9**	
244 ¥ «	**CHEK2 probe** 06636-L30524		**Exon 14**		
250 *	Reference probe 17871-L22467	2p			
256 ¥ « Ж	**CHEK2 probe** 07281-SP0890-L30457		**Exon 15**		
263 *	**ATM probe** 21661-L30283			**Exon 49**	
268 ¥ ¬	**HSCB probe** 06800-L30458		upstream		
274	**CHEK2 probe** 06627-L06185		**Exon 4**		
281 *	**TP53 probe** 21581-L25982				**Exon 1**
286 *	**ATM probe** 21662-L30284			**Exon 17**	
292 *	**ATM probe** 21663-L30525			**Exon 8**	
298 *	Reference probe 15388-L17790	3p			
306 ¥	**CHEK2 probe** 06626-L30841		**Exon 3**		
313 * § Ж ◊ Π	**CHEK2 probe** 22034-SP0468-L31261		**c.1100delC**		
319 *	**ATM probe** 21664-L30286			**Exon 33**	
328 *	**ATM probe** 21665-L30287			**Exon 1**	
337 ◊	**CHEK2 probe** 06624-L24131		**Exon 1**		
346 ¥	**TP53 probe** 00345-L31314				**Exon 11**
355 «	**CHEK2 probe** 19654-L26320		**Exon 13**		
364 *	Reference probe 18676-L24030	11p			
373	**CHEK2 probe** 06628-L06186		**Exon 5**		
382 * «	**CHEK2 probe** 21666-L30288		**Exon 14**		
391	**CHEK2 probe** 06625-L06183		**Exon 2**		
400 « Δ ◊	**CHEK2 probe** 02579-L02041		**Exon 9**		
409	Reference probe 08725-L08736	9q			
418 « Π	**CHEK2 probe** 06632-L06190		**Exon 10**		
427 « Π	**CHEK2 probe** 06631-L06189		**Exon 8**		
436 *	**ATM probe** 21668-L30290			**Exon 5**	
445 *	**ATM probe** 21669-L30291			**Exon 45**	
454 *	Reference probe 18691-L02476	5p			
463 *	**ATM probe** 21670-L30292			**Exon 62**	
474 *	**ATM probe** 21671-L30293			**Exon 29**	
483 *	**ATM probe** 21672-L30294			**Exon 15**	

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	*CHEK2*	*ATM*	*TP53*
490 *	Reference probe 20096-L27538	4p			
500 *	Reference probe 17001-L22947	20q			

[a] See section Exon numbering on page 3 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* c.1100delC mutation is present.

± 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.

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.

¬ 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.

Δ 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.

◊ Detects the same sequence as the CHEK2 probes in SALSA MLPA Probemix P045 BRCA2/CHEK2.

Π Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P056 TP53.

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 aberrations must be confirmed by another method.

Table 3. P190-D1 probes arranged according to chromosomal location

Table 3a. *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
268 ¬	06800-L30458	*HSCB* gene		TGGCTGAAGAAA-TCTGGGTGGACA	1.9 kb
		start codon	*59-61 (Exon 2)*		
220	06623-L31306	Exon 1	208 nt before exon 1	CTAACCAGACTA-ATGTTGCTGATT	0.2 kb
337 ◊	06624-L24131	Exon 1	3-4	TTTAGCGCCACT-CTGCTGGCTGAG	7.3 kb
391	06625-L06183	Exon 2	270-271	CACTCAGGAACT-CTATTCTATTCC	9.2 kb
306	06626-L30841	Exon 3	449-450	AACCACTGCTGA-AAAGAACAGATA	0.2 kb
274	06627-L06185	Exon 4	571-572	ACCTTTGTAAAT-ACAGAGCTTGTA	5.6 kb
373	06628-L06186	Exon 5	704-705	ATCCTAAGGCAT-TAAGAGATGAAT	7.5 kb
208	06629-L30453	Exon 6	780-781	TTTCGAGAGGAA-AACATGTAAGAA	2.0 kb
154	06630-L07119	Exon 7	871-872	CTCAATGTTGAA-ACAGAAATAGAA	6.5 kb
427 « Π	06631-L06189	Exon 8	923-924	TCATCAAGATTA-AAAACTTTTTTG	3.6 kb
400 « Δ ◊	02579-L02041	Exon 9	994-995	CTGTTTGACAAA-GTGGTGGGGAAT	2.9 kb
418 « Π	06632-L06190	Exon 10	1079-1080	ACCTTCATGAAA-ACGGTATTATAC	1.1 kb
313 § Ж ◊ Π	22034-SP0468-L31261	c.1100delC in exon 11	1159-1157 and 38 nt before exon 11, reverse	TGCCCAAAATCA-42 nt spanning oligo-CATAAAATAAAA	0.2 kb
172 « #	21418-L29915	Exon 11	21 nt after exon 11, reverse	ACCAGTCTGTGC-AGCAATGAAAAT	0.6 kb
143 « #	21419-L29916	Exon 12	46 nt after exon 12, reverse	ACCACAGCACAT-ACACATTTTAGC	0.9 kb
355 « #	19654-L26320	Exon 13	54 nt before exon 13	TCTGGCATACTC-TTACTGATAATA	0.1 kb
178 « ± Π #	21654-L30276	Exon 13	1509-1510	CTTAAGACACCC-GTGGCTTCAGGT	4.7 kb
382 « #	21666-L30288	Exon 14	96 nt before exon 14	TGGACGGACATT-TTTCCTCCCTCT	0.4 kb
244 « #	06636-L30524	Exon 14	207 nt after exon 14, reverse	CTGTGCTTATCG-GTCTATTATGTG	1.0 kb
256 « Ж #	07281-SP0890-L30457	Exon 15	1624-1625 and 1666-1667	CGAAAGCGGCCC-42 nt spanning oligo-GCTGTGTGTGCT	
		stop codon	*1688-1690 (Exon 15)*		

Table 3b. *ATM*

Length (nt)	SALSA MLPA probe	*ATM* exon[a]	Ligation site NM_000051.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*151-153 (Exon 2)*		
328	21665-L30287	Exon 1	63 nt before exon 1	GGCTGCTTGGCG-TTGCTTCTTCCT	12.8 kb
436	21668-L30290	Exon 5	579-580	GATTGTAGCAAC-ATACTACTCAAA	11.2 kb
292	21663-L30525	Exon 8	1082-1083	ATGGAGAAGTAT-TTTATACAACTT	2.1 kb
238	21660-L30282	Exon 9	1340-1341	GGAAGTAATAAA-AGATCACCTTCA	8.5 kb
483	21672-L30294	Exon 15	2422-2423	AATGTGCAGGAG-AAAGTATCACTC	9.8 kb
286	21662-L30284	Exon 17	2732-2733	ATTTAACGATTA-CCCTGATAGTAG	4.0 kb
232	21659-L30281	Exon 20	3126-3127	ACTATTTTAAAC-CATGTCCTTCAT	9.8 kb
196	21657-L30279	Exon 24	3699-3700	GTGAAAGAGAAT-GGATTAGAACCT	8.5 kb
474	21671-L30293	Exon 29	4393-4394	TCTAGGATTCCT-ATCAGAAAATTC	7.7 kb
319	21664-L30286	Exon 33	5090-5091	TATGGTGAAACT-AGTTGTCAATTT	10.6 kb
160	21652-L30274	Exon 38	5885-5886	AATGCTTGCTGT-TGTGGACTACAT	9.5 kb
136	21651-L30273	Exon 43	6469-6470	CATGGAGGAATA-TGCAGTGGGACC	3.8 kb
445	21669-L30291	Exon 45	6634-6635	TGTGTAAGCGCA-GCCTTGAGTCTG	7.7 kb
263	21661-L30283	Exon 49	7259-7260	AGTTGCTGGAAA-TTATGATGGAGA	6.0 kb
184	21655-L30277	Exon 55	8265-8266	ATAGATTGTGTA-GGTTCCGATGGC	10.8 kb
131	21650-L30272	Exon 58	8698-8699	TTGAGAAGCGAT-TGGCTTATACGC	9.0 kb
202	21658-L30280	Exon 61	8990-8991	AACTCTGTTAAC-CATTGTAGAGGT	10.3 kb

Length (nt)	SALSA MLPA probe	*ATM* exon[a]	Ligation site NM_000051.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
463	21670-L30292	Exon 62	9035-9036	TGACTGGACCAT-GAATCCTTTGAA	0.3 kb
190	21656-L30840	Exon 63	9279-9280	ATAGACCCCAAA-AATCTCAGCCGA	
		stop codon	*9319-9321 (Exon 63)*		

Table 3c. *TP53*

Length (nt)	SALSA MLPA probe	*TP53* exon[a]	Ligation site NM_000546.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*143-145 (Exon 2a)*		
281	21581-L25982	Exon 1	9 nt before exon 1, reverse	GAGAAGCTCAAA-ACTTTTAGCGCC	11.2 kb
226	01997-L31312	Exon 3	241-242	CATCTACAGTCC-CCCTTGCCGTCC	2.7 kb
166	21653-L30275	Exon 8	1124-1125	ATGGAGAATATT-TCACCCTTCAGG	3.9 kb
346	00345-L31314	Exon 11	1269-1270	AAAGGGTCAGTC-TACCTCCCGCCA	
		stop codon	*1322-1324 (Exon 11)*		

[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.

§ Mutation-specific probe. This probe will generate a signal when the *CHEK2* c.1100delC mutation is present.
± 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.

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.

¬ 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.

Δ 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.

◊ Detects the same sequence as the CHEK2 probes in SALSA MLPA Probemix P045 BRCA2/CHEK2.

Π Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P056 TP53.

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 aberrations must be confirmed by another method.

Related SALSA MLPA probemixes

P002 BRCA1	Contains probes for *BRCA1*, involved in breast and ovarian cancer.
P087 BRCA1 Confirmation	Contains probes for *BRCA1*, as confirmation of the P002 probemix.
P045 BRCA2/CHEK2	Contains probes for *BRCA2* and *CHEK2*, involved in breast and ovarian cancer.
P090 BRCA2	Contains probes for *BRCA2*, involved in breast and ovarian cancer.
P077 BRCA2 Confirmation	Contains probes for *BRCA2*, as confirmation of the P090/P045 probemixes.
P041/P042 ATM	Contain probes for the *ATM* gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for the *TP53* gene, involved in Li-Fraumeni syndrome.
P260 PALB2-RAD50-RAD51C-RAD51D	Contains probes for *PALB2, RAD50, RAD51C* and *RAD51D*, involved in breast and ovarian cancer.

References

- Apostolou P et al. (2018). Characterization and prevalence of two novel CHEK2 large deletions in Greek breast cancer patients. *J Hum Genet*. 63:877-86.
- Buys SS et al. (2017). A study of over 35,000 women with breast cancer tested with a 25-gene panel of hereditary cancer genes. *Cancer*. 123:1721-30.
- Cybulski C et al. (2006). A large germline deletion in the Chek2 kinase gene is associated with an increased risk of prostate cancer. *J Med Gen.* 43:863-6.
- Cybulski C et al. (2007). A deletion in CHEK2 of 5,395 bp predisposes to breast cancer in Poland. *Breast Cancer Res Treat.* 102:119-22.
- Havranek O et al. (2015). Association of germline CHEK2 gene variants with risk and prognosis of non-Hodgkin lymphoma. *PLoS One.* 10:e0140819.
- Micol R et al. (2011). Morbidity and mortality from Ataxia-Telangiectasia are associated with ATM genotype. *J Allergy Clin Immunol*. 128:382-9. E381.
- Mouchawar J et al. (2010). Population-based estimate of the contribution of TP53 mutations to subgroups of early-onset breast cancer: Australian Breast Cancer Family Study. *Cancer Res*. 70:4795-800.
- 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.
- Pan S et al. (2017). Apparent gene conversion event detected in CHEK2 using next generation sequencing Analysis. *Poster 156 at Annual Clinical Genetics Meeting 2017.*
- Podralska MJ et al. (2014). Ten new ATM alterations in Polish patients with Ataxia-Telangiectasia. *Mol Genet Genomic Med*. 2:504-11.
- Ruijs MW et al. (2009). The contribution of CHEK2 to the TP53-negative Li-Fraumeni phenotype. *Hered Cancer Clin Pract.* 7:4.
- Ruijs MW et al. (2010). TP53 germline mutation testing in 180 families suspected of Li-Fraumeni syndrome: mutation detection rate and relative frequency of cancers in different familial phenotypes. *J Med Genet.* 47:421-8.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schulz E et al. (2012). Germline mutations in the DNA damage response genes BRCA1, BRCA2, BARD1 and TP53 in patients with therapy related myeloid neoplasms. *J Med Genet*. 49:422-8.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- 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.
- Tedaldi G et al. (2014). First evidence of a large CHEK2 duplication involved in cancer predisposition in an Italian family with hereditary breast cancer. *BMC Cancer*. 14:478.
- 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.
- 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.
- Walsh T et al. (2006). Spectrum of mutations in BRCA1, BRCA2, CHEK2 and TP53 in families at high risk of breast cancer. *JAMA*. 295:1379-88.
- Xiang HP et al. (2011). Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility. *Eur J Cancer.* 47:2546-51.
- Zhang S et al. (2008). Frequency of the CHEK2 1100delC mutation among women with breast cancer: an international study. *Cancer Res*. 68:2154-7.

Selected publications using SALSA MLPA Probemix P190 CHEK2

- Apostolou P et al. (2021). CHEK2 pathogenic variants in Greek breast cancer patients: evidence for strong associations with estrogen receptor positivity, overuse of risk-reducing procedures and population founder effects. *Cancers (Basel).* 13:2106.

- Fostira F et al. (2020). One in three highly selected Greek patients with breast cancer carries a loss-of-function variant in a cancer susceptibility gene. *J Med Genet.* 57:53-61.
- Havranek O et al. (2015). Association of germline CHEK2 gene variants with risk and prognosis of non-Hodgkin lymphoma. *PloS One.* 10: e0140819.
- Hoyer J et al. (2018). Addition of triple negativity of breast cancer as an indicator for germline mutations in predisposing genes increases sensitivity of clinical selection criteria. *BMC Cancer.* 18:926.
- Judkins T et al. (2015). Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk. *BMC Cancer*. 15:215.
- Kraus C et al. (2017). Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. *Int J Cancer.* 140:95-102.
- Nizic-Kos et al. (2021). Bilateral disease common among Slovenian CHEK2-positive breast cancer patients. *Ann Surg Oncol.* 28:2561-70.
- Novak DJ et al. (2008). Identification of a novel CHEK2 variant and assessment of its contribution to the risk of breast cancer in French Canadian women. *BMC Cancer.* 8:239.
- Rolfes M et al. (2022). Prevalence of cancer predisposition germline variants in male breast cancer patients: Results of the German Consortium for Hereditary Breast and Ovarian Cancer. *Cancers (Basel).* 14:3292.
- Ruijs MW et al. (2009). The contribution of CHEK2 to the TP53-negative Li-Fraumeni phenotype. *Hered Cancer Clin Pract.* 7:4.
- Tedaldi G et al. (2014). First evidence of a large CHEK2 duplication involved in cancer predisposition in an Italian family with hereditary breast cancer. *BMC Cancer*. 14:478.
- Tsaousis GN et al. (2019). Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. *BMC Cancer.* 19:535.
- Vargas-Parra G et al. (2020). Comprehensive analysis and ACMG-based classification of CHEK2 variants in hereditary cancer patients. *Hum Mutat.* 41:2128-42.

P190 product history	
Version	*Modification*
D1	Two CHEK2 probes have been added, the CHEK2 c.1100delC mutation-specific probe has been replaced, all ATM probes have been replaced and twelve have been added, and two TP53 probes have been added. The PTEN, KLLN, XBP1 and BRCA1 probes have been removed. Moreover, almost all reference probes have been replaced and two have been added. Finally, several probes have a small change in length but no change in sequence detected.
C1	One ATM probe has been removed, several probes have been adjusted and four reference probes have been replaced.
B2	New control fragments have been added (QDX2) and three probes have been elongated with no change in hybridising sequence.
B1	All reference probes have been replaced. The BRCA2 probe at 136 nt has been removed.
A1	First release.

Implemented changes in the product description
Version D1-06 – 08 November 2022 (04P) - Link to *TP53* mutation database updated. - Salt sensitivity remark added to CHEK2 exon 8-14 probes (Table 2 and 3a). - Selected publications updated. - Multiple minor textual and layout changes. Version D1-05 – 19 November 2021 (04P) - Product description adapted to a new template. - LRG for *CHEK2* added.

- Coriell sample HG01872 added to positive samples.
- Salt sensitivity remark added to 244 nt and 256 nt probes (Table 2 and 3) and removed from 355 nt probe based on most recent quality tests.
- Ligation sites of the probes targeting the *ATM* and *TP53* genes updated according to new version of the NM_ reference sequences.
- Reference LaBreche et al. (2017) removed.
- Selected publications updated.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version D1-04 – 04 May 2021 (02P)
- Warning Δ corrected below Table 2 and Table 3, for 400 nt CHEK2 probe 02579-L02041.

Version D1-03 – 22 October 2020 (02P)
- Product description adapted to a new template.
- The intended use/purpose has been updated.
- Information about interpretation of signals obtained with the CHEK2 c.1100delC mutation-specific probe added.
- Links to mutation databases updated.
- Warnings added in Table 2 and 3 to the 355 nt and 400 nt probes about salt sensitivity and depurination insensitivity, respectively.
- Various minor textual and layout changes.

Version D1-02 – 27 April 2020 (04)
- Product is now registered for IVD use in Israel.

Version D1-01 – 05 October 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 2 and Table 3).
- Clinical background was rewritten and Table 1 was added.
- SD binning DNA has changed.
- Warning for SNP for probe 07281-SP0890-L30457 was removed.
- Ligation sites of the probes targeting the *CHEK2* gene updated according to new version of the NM_reference sequence.
- Warning added to Table 3 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200

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