Product Description
SALSA® MLPA® Probemix P045-D1 BRCA2/CHEK2

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 13.

Catalogue numbers:
- **P045-025R:** SALSA MLPA Probemix P045 BRCA2/CHEK2, 25 reactions.
- **P045-050R:** SALSA MLPA Probemix P045 BRCA2/CHEK2, 50 reactions.
- **P045-100R:** SALSA MLPA Probemix P045 BRCA2/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).

There are three probemixes available for *BRCA2* testing at MRC Holland. Content and use is described below:

SALSA MLPA Probemix	Target gene(s)	Coverage	Use for	Remarks
P045 BRCA2/CHEK2	*BRCA2*, *CHEK2*	*BRCA2:* Each exon *CHEK2:* Exon 1, 9, c.1100delC mutation (exon 11)	Initial testing by MLPA	All BRCA2 probes are identical to those present in P090 BRCA2.
P090 BRCA2	*BRCA2*	*BRCA2:* Each exon	Initial testing by MLPA	All BRCA2 probes are identical to those present in P045 BRCA2/CHEK2.
P077 BRCA2 Confirmation	*BRCA2*	*BRCA2:* Each exon	Confirmation of MLPA results	The ligation sites of all P077 BRCA2 probes differ from those targeted by P045/P090 BRCA2.

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 P045 BRCA2/CHEK2 is an in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative assay[2] for the detection of deletions or duplications in the *BRCA2* gene and the presence of the wildtype sequence of the *BRCA2* c.156_157insAlu mutation in genomic DNA isolated from human peripheral whole blood specimens. P045 BRCA2/CHEK2 is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome, and, in rare cases, Fanconi Anemia type D1. In addition, deletions and duplications of *CHEK2* exon 1 and exon 9 as well as the presence of the *CHEK2* c.1100delC mutation can be detected with this probemix in order to confirm a potential cause for breast cancer and other *CHEK2*-related cancer types. This product can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P045 BRCA2/CHEK2 should be confirmed with the SALSA MLPA Probemix P077 BRCA2 Confirmation or a different technique. P077 BRCA2 Confirmation cannot be used to verify *CHEK2* mutations. However, the P190 CHEK2 probemix is available for deletion or duplication analysis of all *CHEK2* exons. In particular, CNVs detected by only a single probe always require confirmation by another

method. Most defects in the *BRCA2* and *CHEK2* genes are point mutations, the majority of which will not be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations, 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, SALSA Binning DNA SD067 and Coffalyser.Net analysis software.

Clinical background

Breast and ovarian carcinomas are among the most common malignancies in developed countries. The majority of cases are considered sporadic, but in a substantial portion, a clear history of cases within a family is present. The BRCA1 and BRCA2 proteins are associated with the activation of double-strand break repair and homologous recombination and are important in maintaining genomic stability. Germline mutations in the *BRCA1* and *BRCA2* genes are linked to a high risk of young-onset hereditary breast and ovarian cancer. Features characteristic for hereditary, versus sporadic, breast cancer are: younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of diseases such as prostate and breast cancer among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20-25% of hereditary breast cancers (Easton 1999) and about 5-0% of all breast cancers (Campeau et al. 2008). In addition, mutations in the *BRCA1* and *BRCA2* genes cause around 15% of ovarian cancers (Pal et al. 2005). Women with a germline *BRCA2* mutation have a 45-62% lifetime risk of developing breast cancer, while the risk of women in the general population is 12%. The lifetime risk of developing ovarian cancer in women with a germline *BRCA2* mutation is 11-17%, compared to 1-2% in the general population. Deletions and duplications are more frequent for *BRCA1* than for *BRCA2* in most populations. CNVs in *BRCA2* account for 2-3% of all pathogenic *BRCA2* mutations, dependent on the population. More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

Biallelic pathogenic variants of *BRCA2* can result in Fanconi Anemia (FA) type D1. FA is characterized by physical abnormalities (such as short stature or abnormal skin pigmentation), bone marrow failure and increased risk for malignancies. The incidence of FA in general is 1:160,000, of which type D1 comprises around 3% of the cases. FA type D1 is associated with early-onset acute leukaemia and solid tumours. More information on FA is available at https://www.ncbi.nlm.nih.gov/books/NBK1401/.

BRCA1 and *BRCA2* mutations are the most frequent aberrations found, but other genes are also associated with an increased risk for developing breast and ovarian cancer, including *CHEK2*. The protein CHK2 is a cell cycle checkpoint regulator and a putative tumour suppressor. In non-*BRCA1/2* breast cancer families, patients heterozygous for the *CHEK2* c.1100delC mutation have a two times increased risk of developing breast cancer and have a higher contralateral breast cancer rate (Huijts et al. 2014, Kriege et al. 2014). A deletion of exon 9 and 10 in *CHEK2* has been found mainly in Slavic populations and is associated with a two times higher risk for breast cancer (Walsh et al. 2006).

Gene structure

The *BRCA2* gene spans ~84 kilobases (kb) on chromosome 13q13.1 and contains 27 exons. The *BRCA2* LRG_293 is available at www.lrg-sequence.org and is identical to GenBank NG_012772.3.

The *CHEK2* gene spans ~54 kb on chromosome 22q12.1 (reverse strand) and contains 15 exons. The *CHEK2* LRG_302 is available and is identical to GenBank NG_008150.2.

Transcript variants
For *BRCA2,* one transcript variant has been described encoding the full length protein (NM_000059.4; 11954 nt; coding sequence 200-10456; http://www.ncbi.nlm.nih.gov/gene/675). 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 is located in exon 27.

For *CHEK2,* multiple variants have been described (https://www.ncbi.nlm.nih.gov/gene/11200). Transcript variant 1 is the most predominant 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.

Exon numbering
The *BRCA2* exon numbering used in this P045-D1 BRCA2/CHEK2 product description is the exon numbering from the LRG_293 sequence. The *CHEK2* exon numbering is the exon numbering from the LRG_302 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 P045-D1 BRCA2/CHEK2 contains 51 MLPA probes with amplification products between 130 and 500 nucleotides (nt). This includes 40 probes for the *BRCA2* region and three probes for the *CHEK2* region. At least one MLPA probe is present for each exon in the *BRCA2* transcript; two probes are present for exons 1 and 3, three probes are present for exons 10 and 27, and six probes are present for exon 11. One of the probes for exon 3 detects the wildtype sequence of the c.156_157insAlu mutation and a reduced signal can point towards the presence of this mutation **or** a (partial) deletion of exon 3. In addition, a probe detecting a sequence upstream and a probe detecting a sequence downstream of the *BRCA2* gene are present to determine the extent of a deletion or duplication.

For the *CHEK2* gene, one probe is present for exons 1 and 9. Moreover, one probe specific for the *CHEK2* c.1100delC mutation is included, which will only generate a signal when the mutation is present.

In addition, eight reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA from human 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 HBOC syndrome, Fanconi Anemia type D1 or *CHEK2*-related cancer types. 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 NA03330, NA02718 and HG00187 from the Coriell Institute have been tested with this P045-D1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Coriell Sample ID	Altered target genes in P045-D1	Expected result
NA03330	*BRCA2*	Complete heterozygous *BRCA2* gene duplication
NA02718	*BRCA2*	Complete heterozygous *BRCA2* gene deletion
HG00187	*CHEK2*	Positive for *CHEK2* c.1100delC mutation

SALSA Binning DNA SD067
The SD067 Binning DNA provided with this probemix can be used for binning of all probes including the *CHEK2* c.1100delC mutation-specific probe (490 nt probe 01772-L01336). SD067 Binning DNA is a mixture of genomic DNA from healthy individuals and plasmid DNA that contains the target sequence detected by the above mentioned probe. Inclusion of one reaction with 5 µl SD067 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signal(s). It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD067 Binning DNA product description, available online: www.mrcholland.com.

SALSA Artificial Duplication DNA SD024
In case no positive DNA sample is available in your laboratory, an artificial duplication DNA sample for this probemix (catalogue number SD024) can be ordered from MRC Holland. This SD024 Artificial Duplication DNA will show a duplication of two or more probes when using the following probemixes: P045, P090 and P077 BRCA2; P002 and P087 BRCA1. The SD024 Artificial Duplication DNA is a mixture of human female genomic DNA and a titrated amount of plasmid containing selected probe target sequences. For further details, please

consult the SD024 Artificial Duplication DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Performance characteristics
The expected number of *BRCA2* CNVs that can be detected with this MLPA probemix is between 2 and 3% of all *BRCA2* pathogenic mutations, dependent on the population. The *BRCA2* c.156_157insAlu mutation is a founder mutation of Portuguese origins; the diagnostic sensitivity is ethnicity dependent (Machado et al. 2007, Peixoto et al. 2009). No germline deletions or duplications for *BRCA2* have been described for FA type D1. Deletions or duplications in *CHEK2* are rare, whereas the overall prevalence of the *CHEK2* c.1100delC mutation in breast cancer is around 0.9%, depending on ethnicity (Zhang et al. 2008). The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA2* gene and *CHEK2* exons 1 and 9, the wildtype sequence of the *BRCA2* c.156_157insAlu mutation and the *CHEK2* c.1100delC mutation, is very high and can be considered >99% (based on a 2010-2022 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 the *BRCA2* region and *CHEK2* gene specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), and occasionally 4 (homozygous duplication or heterozygous triplication, e.g. Judkins et al. 2012). A homozygous deletion (copy number 0) of the *BRCA2* gene is unlikely, but may result in FA type D1 or be embryonically lethal (Loizidou et al. 2016).

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 do not apply to the *CHEK2* c.1100delC mutation-specific probe. The peak of the mutation-specific probes 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.

P045 specific notes:

- A hereditary predisposition to breast/ovarian cancer due to *BRCA2* gene defects is an autosomal dominant disorder. Inactivation of a single gene copy of the *BRCA2* gene is thus expected to be pathogenic.
- A duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. Duplication of the *complete BRCA2* gene is not expected to be pathogenic, as it does not cause recombination deficiency (Aref-Eshghi et al. 2020).
- **CHEK2 c.1100delC 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. Please note that this probe will also generate a signal in the unlikely situation that the mutation is present in the *CHEK2* pseudogene. Results obtained with this CHEK2 mutation-specific probe should therefore be treated with caution.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BRCA2* and *CHEK2* genes are small (point) mutations, of which only the *CHEK2* c.1100delC mutation will be detected by using SALSA MLPA Probemix P045 BRCA2/CHEK2.
- 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.
- Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results (see limitation above). This includes the *BRCA2* 999del5 (rs80359671) Finnish/Icelandic founder mutation in *BRCA2* exon 9 (Hartikainen et al. 2007).
- The CHEK2 c.1100delC mutation-specific probe is only intended to determine the presence (or absence) of the mutation.

Confirmation of results

Copy number changes detected with the P045 BRCA2/CHEK2 probemix should be confirmed. The SALSA MLPA Probemix P077 BRCA2 Confirmation can be used for initial confirmation of results. All probes included in SALSA MLPA Probemix P077 BRCA2 Confirmation are different from those in the P045 BRCA2/CHEK2 or P090 BRCA2 probemixes. Alternatively, copy number changes can 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 c.156_157insAlu mutation must be verified with another method, such as nested PCR (Machado et al. 2007). The SALSA MLPA Probemix P190 CHEK2 can be used to further analyse potential deletions and duplications in the *CHEK2* gene. The presence of the *CHEK2* c.1100delC mutation should always be confirmed by sequence analysis.

Copy number changes detected by only a single probe always require confirmation with SALSA MLPA Probemix P077 BRCA2 Confirmation or by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

***BRCA2/CHEK2* mutation databases**

For *BRCA2*: https://databases.lovd.nl/shared/genes/BRCA2.
For *CHEK2*: https://databases.lovd.nl/shared/genes/CHEK2.
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 *BRCA2* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P045-D1 BRCA2/CHEK2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*BRCA2*	*CHEK2*
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 00797-L00463	5q		
136	**BRCA2 probe** 02283-L26707		**Exon 1**	
142	**BRCA2 probe** 18385-L23778		**Exon 11**	
149	**BRCA2 probe** 20546-L28140		**Exon 19**	
154	**BRCA2 probe** 02285-L23744		**Exon 1**	
160	**BRCA2 probe** 09297-L28129		**Exon 14**	
166	**BRCA2 probe** 20603-L28261		**Exon 11**	
172	**BRCA2 probe** 02486-L23747		**Exon 2**	
178	Reference probe 04532-L03921	2q		
184	**BRCA2 probe** 20625-L28317		**Exon 22**	
190	**BRCA2 probe** 18387-L24251		**Exon 11**	
196	**BRCA2 probe** 09812-L23750		**Exon 23**	
202	**BRCA2 probe** 01600-L23751		**Exon 4**	
208	**BRCA2 probe** 08265-L23752		**Exon 7**	
214	Reference probe 11996-L12824	6q		
220	**BRCA2 probe** 18388-L23375		**Exon 10**	
226	**BRCA2 probe** 20626-L28778		**Exon 25**	
232	**BRCA2 probe** 01603-L13850		**Exon 9**	
238 ∞	**BRCA2 probe** 22219-L31553		**Exon 3; c.156_157insAlu**	
244 ¬	Flanking probe20548-L31554		Upstream	
250	**BRCA2 probe** 01604-L23754		**Exon 10**	
257	Reference probe 02469-L28780	15q		
265	**BRCA2 probe** 20549-L28781		**Exon 11**	
271 »	**CHEK2 probe** 20724-L29194			**Exon 1**
275	**BRCA2 probe** 18389-L24255		**Exon 27**	
283	**BRCA2 probe** 01606-L23757		**Exon 11**	
291	**BRCA2 probe** 20676-L28319		**Exon 18**	
295	**BRCA2 probe** 20541-L28782		**Exon 27**	
304	Reference probe 11441-L28327	1q		
313	**BRCA2 probe** 02280-L28326		**Exon 13**	
321	**BRCA2 probe** 09809-L28325		**Exon 5**	
328	**BRCA2 probe** 19699-L28324		**Exon 27**	
337	**BRCA2 probe** 20628-L28320		**Exon 12**	
346	**BRCA2 probe** 01611-L23763		**Exon 16**	
355	**BRCA2 probe** 04585-L23764		**Exon 6**	
364	**BRCA2 probe** 02281-L23765		**Exon 17**	
373	**BRCA2 probe** 20629-L28321		**Exon 21**	
382	Reference probe 13329-L14755	18q		
391	**BRCA2 probe** 20543-L28130		**Exon 10**	
400	**BRCA2 probe** 08266-L23768		**Exon 20**	
409 « » Ж	**CHEK2 probe** 02579-L23769			**Exon 9**
418	**BRCA2 probe** 20630-L28322		**Exon 15**	
426	**BRCA2 probe** 20631-L25993		**Exon 3**	
436	Reference probe 07975-L07756	17q		
445	**BRCA2 probe** 08267-L23772		**Exon 24**	
454	**BRCA2 probe** 20632-L28323		**Exon 8**	
462 ¬	Flanking probe 18948-L01619		Downstream	
472	**BRCA2 probe** 11984-L23775		**Exon 26**	
481	**BRCA2 probe** 20550-L28144		**Exon 11**	
490 § »	**CHEK2 probe** 01772-L01336			**Exon 11; c.1100delC**
500	Reference probe 21229-L29604	10p11		

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

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

∞ Wild type sequence detected. A lowered probe signal can be due to a (partial) *BRCA2* exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

« 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.

¬ 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.

» Detects the same sequence as the CHEK2 probes in SALSA MLPA Probemix P190 CHEK2.

Ж A high signal of the 409 nt 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 a decreased signal for this probe in the test samples. The 232 nt probe (01603-L13850) can show a similar trend, whereas probes at 130 nt (00797-L00463), 149 nt (20546-L28140) and 166 nt (20603-L28261) will show the opposite trend. Please consult the Support section on www.mrcholland.com for more information on depurination.

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

Table 2. P045-D1 probes arranged according to chromosomal location

Table 2a. *BRCA2*

Length (nt)	SALSA MLPA probe	*BRCA2* exon[a]	Ligation site NM_000059.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
244 ¬	20548-L31554	Upstream		AGAGAACAAGAA-ACATAAAGGTAT	1.7 kb
136	02283-L26707	Exon 1	28 nt before exon 1	CAGCGCGGGCTT-GTGGCGCGAGCT	0.2 kb
154	02285-L23744	Exon 1	23 nt after exon 1	TGGTAGTGGGTT-GGGACGAGCGCG	0.8 kb
		start codon	*200-202 (Exon 2)*		
172	02486-L23747	Exon 2	243-242 reverse	AGCGTGTCTTAA-AAATTTCAAAAA	2.7 kb
238 ∞	22219-L31553	Exon 3	353-354 WT at c.156_157insAlu	AAGAATCTGAAC-ATAAAAACAACA	0.1 kb
426	20631-L25993	Exon 3	444-445	AATAATATTCAA-AGAGCAAGGGCT	5.9 kb
202	01600-L23751	Exon 4	541-542	AATAGTAGACAT-AAAAGTCTTCGC	1.0 kb
321	09809-L28325	Exon 5	660-661	TGTAACACCACA-AAGAGATAAGTC	0.1 kb
355	04585-L23764	Exon 6	700-699 reverse	ACAAACTTTGGT-GTATGAAACAAA	0.3 kb
208	08265-L23752	Exon 7	784-785	ATGTCTTGGTCA-AGTTCTTTAGCT	2.9 kb
454	20632-L28323	Exon 8	865-864 reverse	GTAGTATCATGA-GGAAATACAGTT	1.5 kb
232	01603-L13850	Exon 9	973-974	AACACAAATCAA-AGAGAAGCTGCA	1.6 kb
250	01604-L23754	Exon 10	1346-1347	GAAGTGACAAAA-TCTCCAAGGAAG	0.5 kb
220	18388-L23375	Exon 10	1886-1885 reverse	GGTGGCTGGCCA-GCTTCCATTATC	0.2 kb
391	20543-L28130	Exon 10	2076-2077	AAATGCTTTTGA-AGCACCACTTAC	3.0 kb
265	20549-L28781	Exon 11	2216-2215 reverse	ACATGTTTCATT-TCTAGAACATTT	1.0 kb
142	18385-L23778	Exon 11	3221-3222	GTTTTGGAGGTA-GCTTCAGAACAG	0.7 kb
166	20603-L28261	Exon 11	3927-3926 reverse	TATTCTCAATAT-CACTAAACAGTT	1.3 kb
190	18387-L24251	Exon 11	5191-5190 reverse	GCTGAATTTTCA-ATGACTGAATAA	1.1 kb
481	20550-L28144	Exon 11	6245-6246	CCAAAGTATTGT-TTAAAAGTAACG	0.7 kb
283	01606-L23757	Exon 11	6964-6965	TCTCTTTTTACA-TGTCCCGAAAAT	3.5 kb
337	20628-L28320	Exon 12	7126-7127	GCTTCAAAAAGC-ACTCCAGATGGT	2.2 kb
313	02280-L28326	Exon 13	7188-7187 reverse	GTACACAGGTAA-TCGGCTCTAAAG	8.2 kb
160	09297-L28129	Exon 14	7366-7367	TCTGCTACAAGA-AATGAAAAAATG	1.5 kb
418	20630-L28322	Exon 15	7734-7735	CAGTCTGTATCT-TGCAAAAACATC	1.4 kb
346	01611-L23763	Exon 16	7947-7948	ACAGTTGGCTGA-TGGTGGATGGCT	4.8 kb
364	02281-L23765	Exon 17	8130-8129 reverse	TTAGGCATCTAT-TAGCAAATTCCT	0.8 kb
291	20676-L28319	Exon 18	8454-8455	TCAGAAGATTAT-TCTTCATGGAGC	7.0 kb

149	20546-L28140	Exon 19	8590-8591	TTCTTTCCTGAC-CCTAGACCTTTT	0.5 kb
400	08266-L23768	Exon 20	8715-8716	ATCTGGATTATA-CATATTTCGCAA	5.7 kb
373	20629-L28321	Exon 21	8881-8882	ACAAGACAGCAA-GTTCGTGCTTTG	2.7 kb
184	20625-L28317	Exon 22	9072-9073	TGCTGAACAAAA-GGAACAAGGTTT	0.3 kb
196	09812-L23750	Exon 23	9186-9187	ATCATCAGATTT-ATATTCTCTGTT	0.3 kb
445	08267-L23772	Exon 24	9427-9426 reverse	GAAACGACAAAT-CCTATTAGGTCC	14.8 kb
226	20626-L28778	Exon 25	9678-9679	AGAGACATTCAA-CAAAATGAAAAA	2.1 kb
472	11984-L23775	Exon 26	9758-9759	TACTGCATGCAA-ATGATCCCAAGT	1.3 kb
295	20541-L28782	Exon 27	9960-9961	AAAGTCTTGTAA-AGGGGAGAAAGA	0.4 kb
328	19699-L28324	Exon 27	10347-10348	TCTCAGACTGAA-ACGACGTTGTAC	0.8 kb
275	18389-L24255	Exon 27	11111-11110 reverse	GAAACACCACTC-TTCATATTCATC	7.9 kb
		stop codon	*10454-10456 (Exon 27)*		
462 ¬	18948-L01619	Downstream		CATTATTATTGA-TAATACCAACCT	

Table 2b. *CHEK2*

Length (nt)	SALSA MLPA probe	*CHEK2* exon[a]	Ligation site NM_007194.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*59-61 (Exon 2)*		
271 »	20724-L29194	Exon 1	3-4	TTTAGCGCCACT-CTGCTGGCTGAG	41.9 kb
409 « » Ж	02579-L23769	Exon 9	994-995	CTGTTTGACAAA-GTGGTGGGGAAT	4.0 kb
490 » §	01772-L01336	Exon 11	1159-1157 reverse c.1100delC mutation	TGCCCAAAATCA-TAATCTAAAATT	
		stop codon	*1688-1690 (Exon 15)*		

[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 only generate a signal when the *CHEK2* c.1100delC mutation is present.

∞ Wild type sequence detected. A lowered probe signal can be due to a BRCA2 exon 3 deletion or due to the presence of the c.156_157insAlu (Portuguese founder) mutation. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

¬ 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.

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

» Detects the same sequence as one of the CHEK2 probes in SALSA MLPA Probemix P190 CHEK2.

Ж A high signal of the 409 nt 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 a decreased signal for this probe in the test samples. The 232 nt probe (01603-L13850) can show a similar trend, whereas probes at 130 nt (00797-L00463), 149 nt (20546-L28140) and 166 nt (20603-L28261) will show the opposite trend. Please consult the Support section on www.mrcholland.com for more information on depurination.

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

Related SALSA MLPA probemixes

P090 BRCA2	Contains the same probes for the BRCA2 gene as in probemix P045.
P077 BRCA2 Confirmation	Contains probes for the BRCA2 gene. It can be used to confirm the results obtained with probemix P045 or P090.
P190 CHEK2	Contains probes for the CHEK2, ATM and TP53 genes, involved in cancer.
P002 BRCA1	Contains probes for the BRCA1 gene. It should be used for primary screening of BRCA1.

P087 BRCA1 Confirmation	Contains probes for the BRCA1 gene. It can be used to confirm the results obtained with probemix P002.
P239 BRCA1 region	Contains probes for the BRCA1 region. It can be used to characterise deletions/duplications extending upstream or downstream of BRCA1. Four probes in probemix P239 have the same ligation sites as probes present in probemix P002.
P041/P042 ATM	Contain probes for the ATM gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for TP53, involved in Li-Fraumeni syndrome.
P240 BRIP1/CHEK1	Contains probes for the BRIP1 and CHEK1 genes, involved in breast and ovarian cancer.
P260 PALB2-RAD50-RAD51C-RAD51D	Contains probes for the PALB2, RAD50, RAD51C and RAD51D genes, involved in breast and ovarian cancer.

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P045 product history	
Version	*Modification*
D1	The BRCA2 exon 3/c.156_157insAlu probe has been changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One probe has a small change in length, but not in sequence detected. One reference probe has been replaced.
C1	The probes for the *BRCA2* upstream region and exons 8, 11, 12, 19 and 27 have been replaced, and extra probes have been added for longer exons. A probe detecting the *BRCA2* c.156_157insAlu mutation has been included. The BRCA2 exon 3 probe that detects the c.504del5068insCCAT mutation has been removed. For *CHEK2,* the exon 1 probe has been replaced. In addition, most reference probes have been replaced and the lengths of most target probes have been adjusted.
B3	The 88 and 96 nt DNA denaturation control fragments (QDX2) have been replaced.
B2	Four reference probes have been replaced and extra control fragments at 100 and 105 nt (X, Y chromosome specific) have been included.
B1	Three new probes for BRCA2 have been added and seven probes have been replaced as compared to previous versions of this probe set. In addition, two DNA denaturation control probes at 88 and 96 nt are now also included in P045B.
A0	Two extra BRCA2 probes (exons 6 and 26) and a new exon 25 probe has been added as compared to the previous lots 0204, 0804 & 0105.
A	First release.

Implemented changes in the product description

Version D1-05 – 08 June 2023 (04P)
- Product is no longer registered for IVD use in Morocco.

Version D1-04 – 01 December 2022 (04P)
- Table on page 1 updated to clarify that P045/P090 must be used as initial testing by MLPA.
- Sections Clinical background and Performance characteristics updated with new information.
- Clarified that the BRCA2 flanking probes are present to determine the extent of the deletion or duplication in the section Probemix content.
- Clarified that flanking probes are unlikely to have any relation to the condition tested for, in the section Interpretation of results.
- Information about interpretation of signals obtained with the CHEK2 c.1100delC mutation-specific probe added.
- The 244 nt and 462 nt probes denoted as flanking probes in Table 1.

- Salt warning added to Table 1 and 2 for the 409 nt probe.
- Sections References and Selected publications curated.
- Minor textual and layout changes.

Version D1-03 – 06 July 2021 (04P)
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- UK added to the list of countries in Europe that accept the CE mark.
- Gene structure section, Transcript variants section, Interpretation of results section, Performance characteristics section, Confirmation of results section and Mutation database section updated.
- Ligation sites of the probes targeting the *BRCA2* gene updated according to new version of the NM_ reference sequence.
- References in References section and Selected publications section updated.

Version D1-02 – 31 March 2020 (02P)
- The removal of the BRCA2 exon 3/ c.504del5068insCCAT probe in product version C1 was added to the P045 product history.
- Costa Rica was added as country with IVD status.
- Various minor textual or layout changes.

Version D1-01 – 28 May 2019 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Intended use has been adjusted to include FA type D1.
- Information about FA type D1 was added to the clinical background section and performance characteristics section.
- Interpretation of results section concerning homozygous deletions (copy number 0) was updated.
- Warning was added for probe 02579-L23769 in Table 1 and 2b.
- Various minor textual or layout changes.
- Limitation on risk of positive results due to founder mutations was added.
- Reference section of probemixes using P045 updated.

Version C1-04 – 05 October 2018 (04)
- Product description restructured and adapted to a new template.
- Various minor textual or layout changes.
- Updated positive sample section with *CHEK2* 1100delC positive sample.
- Wording for *CHEK2* 1100delC note was adjusted.
- Ligation sites of the probes targeting the *CHEK2* gene updated according to new version of the NM_reference sequence.
- Note was added under Table 1 and 2 for CHEK2 probes that have the same sequence as probes in the P190 probemix.
- Updated the related probemixes section.
- References using probemix P045 were updated.
- Countries where product has IVD status was updated.

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