Instructions for Use

SALSA® MLPA® Probemix P045 BRCA2/CHEK2

See also the MLPA General Protocol, and the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD067, SALSA® Artificial Duplication DNA SD024, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P045 BRCA2/CHEK2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P045 BRCA2/CHEK2**
Version	**D1**
Catalogue numbers	**P045-025R (25 reactions)** **P045-050R (50 reactions)** **P045-100R (100 reactions)**
Basic UDI-DI	**872021148P0455S**
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 SD067	SD067
SALSA® Artificial Duplication DNA SD024 (optional)	SD024

Available *BRCA2* Probemixes

SALSA MLPA Probemix	**Coverage**	**Used for**
P045 BRCA2/CHEK2	*BRCA2**: all exons *CHEK2*: exon 1, 9, c.1100delC mutation (exon 11)	**Initial** testing by MLPA
P090 BRCA2	*BRCA2**: all exons	**Initial** testing by MLPA
P077 BRCA2 Confirmation	*BRCA2*: all exons	**Confirmation** of MLPA results

* Probemix P045 and P090 contain the same probes for the *BRCA2* gene

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 COLOMBIA ISRAEL COSTA RICA
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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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

D1 version compared to C1 version

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.

1. Intended Purpose

The SALSA MLPA Probemix P045 BRCA2/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 *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. 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 and clinical diagnosis of a predisposition to breast cancer and other *CHEK2*-related cancer types in individuals originally suspected of HBOC syndrome. This product can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) in *BRCA2* 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* CNVs or mutations. However, the SALSA MLPA Probemix P190 CHEK2 is available for CNV 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 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 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 HBOC syndrome or predisposition to *CHEK2*-related cancer types.	
No-DNA control (preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Binning DNA sample (initial experiment)	• SALSA Binning DNA SD067, provided by MRC Holland • Recommended 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 at MRC Holland	SALSA® Artificial Duplication DNA SD024 (duplication of five probes, presence of one mutation)
	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 (*BRCA2* and *CHEK2*)

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

Study	Description
Expected values for copy numbers in normal and affected populations	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 P045 BRCA2/CHEK2 in 44 samples from healthy individuals with a normal *BRCA2* and *CHEK2* copy number and nine samples with known *BRCA2* or *CHEK2* CNVs. The expected FRs for the corresponding copy numbers were found in all samples tested.
Expected values for point mutation detection in normal and affected populations	The mutation-specific probe will only generate a signal when the *CHEK2* c.1100delC mutation is present. Please note that background signals of the mutation-specific probe can be expected above the threshold in some cases. Users should always compare the relative peak height of the mutation-specific probe in mutation-positive samples to the relative peak height in reference samples. A clear signal (at least 10% of the median peak height of all reference probes in that sample) indicates that the mutation is present. It is not possible to determine the copy number of mutation-specific probes. The expected value for mutation-specific probe was verified with P045 using one mutation positive sample, eight samples positive for other *BRCA2* or *CHEK2* aberrations detected by SALSA MLPA Probemix P045 BRCA2/CHEK2, and 44 samples from healthy individuals without the *CHEK2* c.1100delC mutation, and the expected results were found in all tested samples.
Limit of detection	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 recommended 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 P045 BRCA2/CHEK2 on five samples with known CNVs/mutation status and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.

<table>
<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 P045 BRCA2/CHEK2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/mutation status. For most probes, expected FRs 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 576/600 measurements
Mutation: Expected % for 15/15 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 596/600 measurements
Mutation: Expected % for 15/15 probes</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 597/600 measurements
Mutation: Expected % for 15/15 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 595/600 measurements
Mutation: Expected % for 15/15 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 540/600 measurements
Mutation: Expected % for 15/15 measurements</td></tr>
</table>
* Results are summarised for all BRCA2 and CHEK2 probes across all five samples tested in triplicate.

$FeCl_3$, NaCl 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, in particular for copy number determination. The interferents had no 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>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence. The expected final ratios were obtained from 1118/1134 (98.59%) measurements and all unexpected results fell in the ambiguous range, so no false results were obtained. All probes met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, nine previously genotyped samples were tested using SALSA MLPA Probemix P045 BRCA2/CHEK2 and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutation status over multiple days, and by multiple operators. Results showed a correct call in 252/252 (between replicates), 736/738 (between days) and 736/738 (between operators) measurements, and no false results were obtained, leading to a precision of >99%.</td></tr>
<tr><td>Clinical validity*</td><td>BRCA2: 80% of HBOC syndrome cases are linked to BRCA1 or BRCA2 mutations. Among these, 34% are due to a pathogenic variant in BRCA2. Of these BRCA2 variants, approximately 2-3% are deletions or duplications, which can be detected using gene-targeted deletion/duplication analysis.

The BRCA2 c.156_157insAlu mutation is a founder mutation of Portuguese origin. Therefore, the frequency of BRCA2 c.156_157insAlu mutation varies across populations.

CHEK2: Frequencies of CHEK2 mutations in the general population vary widely per mutation and ethnicity, they are estimated to be 0-3.5%.The CHEK2 c.1100delC mutation is the most common founder mutation of this gene and it is of Northern and Eastern European origin. Therefore, the frequency of CHEK2 c.1100delC mutation varies across populations.

*(Based on a 2000-2023 literature review)</td></tr>
</table>

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
13q13.1	*ZAR1L*		1.7 kb		244	20548-L31554	¬
13q13.1	*BRCA2*	Exon 1	0.2 kb		136	02283-L26707	+
13q13.1	*BRCA2*	Exon 1	0.8 kb		154	02285-L23744	+
13q13.1	*BRCA2*	Exon 2	2.7 kb		172	02486-L23747	
13q13.1	*BRCA2*	Exon 3	0.1 kb	c.156_157insAlu (wildtype)	238	22219-L31553	∞
13q13.1	*BRCA2*	Exon 3	5.9 kb		426	20631-L25993	
13q13.1	*BRCA2*	Exon 4	1.0 kb		202	01600-L23751	
13q13.1	*BRCA2*	Exon 5	0.1 kb		321	09809-L28325	
13q13.1	*BRCA2*	Exon 6	0.3 kb		355	04585-L23764	
13q13.1	*BRCA2*	Exon 7	2.9 kb		208	08265-L23752	
13q13.1	*BRCA2*	Exon 8	1.5 kb		454	20632-L28323	
13q13.1	*BRCA2*	Exon 9	1.6 kb		232	01603-L13850	
13q13.1	*BRCA2*	Exon 10	0.5 kb		250	01604-L23754	
13q13.1	*BRCA2*	Exon 10	0.2 kb		220	18388-L23375	
13q13.1	*BRCA2*	Exon 10	3.0 kb		391	20543-L28130	
13q13.1	*BRCA2*	Exon 11	1.0 kb		265	20549-L28781	
13q13.1	*BRCA2*	Exon 11	0.7 kb		142	18385-L23778	
13q13.1	*BRCA2*	Exon 11	1.3 kb		166	20603-L28261	
13q13.1	*BRCA2*	Exon 11	1.1 kb		190	18387-L24251	
13q13.1	*BRCA2*	Exon 11	0.7 kb		481	20550-L28144	
13q13.1	*BRCA2*	Exon 11	3.5 kb		283	01606-L23757	
13q13.1	*BRCA2*	Exon 12	2.2 kb		337	20628-L28320	
13q13.1	*BRCA2*	Exon 13	8.2 kb		313	02280-L28326	
13q13.1	*BRCA2*	Exon 14	1.5 kb		160	09297-L28129	
13q13.1	*BRCA2*	Exon 15	1.4 kb		418	20630-L28322	
13q13.1	*BRCA2*	Exon 16	4.8 kb		346	01611-L23763	
13q13.1	*BRCA2*	Exon 17	0.8 kb		364	02281-L23765	
13q13.1	*BRCA2*	Exon 18	7.0 kb		291	20676-L28319	
13q13.1	*BRCA2*	Exon 19	0.5 kb		149	20546-L28140	
13q13.1	*BRCA2*	Exon 20	5.7 kb		400	08266-L23768	
13q13.1	*BRCA2*	Exon 21	2.7 kb		373	20629-L28321	
13q13.1	*BRCA2*	Exon 22	0.3 kb		184	20625-L28317	
13q13.1	*BRCA2*	Exon 23	0.3 kb		196	09812-L23750	
13q13.1	*BRCA2*	Exon 24	14.8 kb		445	08267-L23772	
13q13.1	*BRCA2*	Exon 25	2.1 kb		226	20626-L28778	
13q13.1	*BRCA2*	Exon 26	1.3 kb		472	11984-L23775	
13q13.1	*BRCA2*	Exon 27	0.4 kb		295	20541-L28782	
13q13.1	*BRCA2*	Exon 27	0.8 kb		328	19699-L28324	
13q13.1	*BRCA2*	Exon 27	7.9 kb		275	18389-L24255	
13q13.1	*N4BP2L1*				462	18948-L01619	¬
22q12.1	*CHEK2*	Exon 11	4.0 kb	c.1100delC	490	01772-L01336	§ ◊ »
22q12.1	*CHEK2*	Exon 9	41.9 kb		409	02579-L23769	« ∫ »
22q12.1	*CHEK2*	Exon 1			271	20724-L29194	»
1q	Reference				304	11441-L28327	
2q	Reference				178	04532-L03921	
5q	Reference				130	00797-L00463	
6q	Reference				214	11996-L12824	
10p	Reference				500	21229-L29604	
15q	Reference				257	02469-L28780	
17q	Reference				436	07975-L07756	
18q	Reference				382	13329-L14755	

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 *BRCA2* and *CHEK2* exon numbers are derived from the MANE project, and based on MANE Select transcripts. For more information, see the probe sequences document available on the product page at www.mrcholland.com.
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.

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

¬ This 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.

◊	This probe can give an extra signal in all samples due to incomplete ligase inactivation.
»	Detects the same sequence as one of the probes in SALSA® MLPA® Probemix P190 CHEK2.
+	The ligation site of these probes 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.
∫	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 can show a similar trend, whereas probes at 130 nt, 149 nt and 166 nt will show the opposite trend. Please consult the Support section on www.mrcholland.com for more information on depurination.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. 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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The clinical significance of CNVs in *BRCA2* is not clearly established for Fanconi Anemia Type D1.
2. The mutation-specific probe can only detect the presence of the mutation and should not be used to determine zygosity.
3. Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results. This includes the *BRCA2* 999del5 (rs80359671) Finnish/Icelandic founder mutation in exon 9 (Hartikainen et al. 2007).

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Hartikainen JM et al. (2007). Screening for BRCA1 and BRCA2 mutations in Eastern Finnish breast/ovarian cancer families. Clin Genet. 72:311-20.

Implemented changes in the product description

Version D1-07 – 06 August 2025 (03S)
- Reference to SALSA Binning DNA SD067 removed from the intended purpose footnote.
- Binning DNA sample (initial experiment) description rephrased following the removal of SALSA Binning DNA SD067 from the intended purpose.
- Minor textual changes made in the section 6. Performance Characteristics.

Version D1-06 – 24 January 2025 (03S)
- Product description was adapted to a new template.
- Intended purpose was updated, Fanconi Anemia type D1 removed, specifying the testing population and clinical application of *CHEK2* detection, and specifying assay is manual.
- Probemix-specific limitation about the clinical significance of *BRCA2* CNVs in Fanconi Anemia Type D1 was added.
- Warning for the 490 nt probe detecting the *CHEK2* c.1100delC mutation being sensitive to incomplete ligase activation was added.
- Probemix is now IVDR certified.