Instructions for Use

SALSA® MLPA® Probemix P090 BRCA2

See also the MLPA General Protocol, the product description(s) of the SALSA® MLPA® Reagent Kit, SALSA® Artificial Duplication DNA SD024 and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P090 BRCA2 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P090 BRCA2**
Version	C1
Catalogue numbers	P090-025R (25 reactions) P090-050R (50 reactions) P090-100R (100 reactions)
Basic UDI-DI	872021148P0905X
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® 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

* Probemixes P045 BRCA2/CHEK2 and P090 BRCA2 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 MOROCCO
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 or country in which the user and/or the patient is located.

Changes in this Product Version

C1 version compared to B1 version

The exon 3/c.156_157insAlu wildtype probe changed from a 3-part to a 2-part probe in order to reduce its sensitivity to sample DNA depurination. One reference probe removed and one probe has a small change in length but not in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P090 BRCA2 is an in vitro diagnostic (IVD)[1] or a 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. P090 BRCA2 is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome. This product can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P090 BRCA2 should be confirmed with the SALSA MLPA Probemix P077 BRCA2 Confirmation or a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *BRCA2* gene 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
Collection, Storage, and Shipment Methods	• DNA source: standard methods for collection, storage, and shipment for tube-collected blood. • Purified DNA: long-term storage at -15 to -25°C.
Extraction 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. • Derived from unrelated individuals from families without a history of HBOC syndrome. • At least three independent reference samples required in each experiment for proper data normalisation. When testing >21 test samples, additional reference samples should be added: one for every additional seven test samples. See our support portal for more information.	
No-DNA Control (Preferably)	• Provided by user. • $TE_{0.1}$ buffer instead of DNA. • To check for DNA contamination.	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from MRC Holland.	SALSA® Artificial Duplication DNA SD024 (duplication of five probes).
	Available from third parties.	See the table of positive samples on the probemix product page on our website.

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

FR	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*BRCA2*)

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 FR and corresponding copy number. Test results are expected to centre around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a single nucleotide variant (SNV) influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of BRCA2 c.156_157insAlu Wildtype-Specific Probe

238 nt BRCA2 c.156_157insAlu Wildtype-Specific Probe	
FR	**Mutation and Copy Number Status**
0	Homozygous *BRCA2* c.156_157insAlu OR homozygous *BRCA2* exon 3 deletion OR compound heterozygosity of these *BRCA2* mutations
0.40 – 0.65	Heterozygous *BRCA2* c.156_157insAlu mutation OR heterozygous *BRCA2* exon 3 deletion
0.80 – 1.20	*BRCA2* c.156_157insAlu mutation and exon 3 deletion not detected

Wildtype probes detect the wildtype sequence of a mutation. A decreased probe signal can be due to a deletion or the presence of the mutation. The FR of copy number probes close to wildtype probes may help to differentiate between a mutation versus a deletion. The table illustrates the relationship between FR and corresponding mutation and copy number status.

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>To determine the expected values in normal and affected populations, a study was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. Cut-off values for copy number determination were then verified with P090 BRCA2 in 68 samples from healthy individuals with a normal BRCA2 copy number and four samples with known BRCA2 CNVs. The expected FRs for all copy number probes, including the BRCA2 exon 3/c.156_157insAlu wildtype probe, were found in >99% of measurements.</td></tr>
<tr><td>Limit of detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. These lower and higher limits of detection were verified using P090 BRCA2 on two samples with known CNVs. Expected results were obtained for >99% measurements (569/570) in both samples using the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>A study using P090 BRCA2 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on six samples with known copy number. 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 225/228 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 227/228 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 228/228 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 226/228 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 224/228 measurements</td></tr>
</table>
* Results are summarised for all BRCA2 probes across all six samples tested in triplicate. Results for the BRCA2 exon 3/c.156_157insAlu wildtype probe are reported as a copy number probe.

A small number of ambiguous results were obtained in reactions containing EDTA, NaCl, heparin or hemoglobin. None of the substances caused false results, and no effects were observed on FRs with any of the substances.</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 and the expected results were obtained for all measurements (608/608). 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, three previously genotyped samples were tested using P090 BRCA2 and the expected results were obtained for 100% of measurements (304/304). Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 227/228 (99.6%) measurements between replicates, 227/228 (99.6%) measurements between days and 224/228 (98.3%) measurements between operators, and no false results were obtained. Overall, precision is >98%.</td></tr>
<tr><td>Clinical performance*</td><td>Diagnostic sensitivity: The diagnostic sensitivity of P090 BRCA2 is 95.1–99.3%.

Diagnostic specificity: The diagnostic specificity of P090 BRCA2 is 99.4 - 99.8%.

Positive predictive value: The positive predictive value of P090 BRCA2 is estimated to be >99% in the presence of indicative clinical features, i.e. a personal or family history of related cancer. Mutations identified by P090 BRCA2 indicate HBOC syndrome even in the absence of cancer.

Negative predictive value: HBOC syndrome can be caused by numerous SNVs and/or CNVs in other cancer predisposition genes, most of which cannot be detected by P090 BRCA2. However, the probability that an individual with a negative P090 BRCA2 test does not have BRCA2-related HBOC syndrome is high if other types of BRCA2 mutations, such as SNVs, have been ruled out. In this case, the NPV of P090 BRCA2 is >99%.

Likelihood ratio: The likelihood ratio of P090 BRCA2 is 165.5 – 475.5.

* Based on a 2006-2026 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	¬
1q	Reference				304	11441-L28327	
2q	Reference				178	04532-L03921	
3p	Reference				409	15392-L17223	
5p	Reference				269	03075-L20665	
5q	Reference				130	00797-L00463	
6q	Reference				214	11996-L12824	
15q	Reference				257	02469-L28780	
17q	Reference				436	07975-L07756	
18q	Reference				382	13329-L14755	
22q	Reference				490	12461-L21828	

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* exon numbers are derived from the MANE project and are based on the MANE Select transcript. For more information, see the probe sequences document available on the product page. Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

∞ 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.

¬ These probes are flanking probes, 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.

\+ The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page.

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. We have received reports of sample or technical artefacts that may appear as a (mosaic) copy number change of the whole gene. Whole gene deletions or duplications should therefore be confirmed by a different technique and/or analysis of an independent DNA sample to exclude false positive results. For more information on this issue, please contact info@mrcholland.com.
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.

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. Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results. This includes the *BRCA2* c.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

- 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 C1-07 – 02 November 2026 (04S)
- Adapted to new template.
- Possible Results of BRCA2 Wildtype-Specific Probe added to section 5.
- Performance characteristics updated: results obtained with the BRCA2 c.156_157insAlu wildtype probe specified, additional detail regarding the number of measurements added for some analytical performance characteristics, correction made for the number of samples tested for trueness, clinical performance updated, all sections edited for brevity.
- Precaution regarding sample and technical artefacts reworded.

Version C1-06 – 27 October 2025 (03S)
- Probemix is now registered for IVD use in Morocco.

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