Instructions for Use

SALSA® MLPA® Probemix P087 BRCA1 Confirmation

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

Visit the SALSA® MLPA® Probemix P087 BRCA1 Confirmation product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P087 BRCA1 Confirmation**
Version	**D1**
Catalogue numbers	**P087-025R (25 reactions)** **P087-050R (50 reactions)** **P087-100R (100 reactions)**
Basic UDI-DI	**872021148P0876A**
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 *BRCA1* Probemixes

SALSA MLPA Probemix	**Coverage**	**Used for**
P002 BRCA1	*BRCA1*: all exons	**Initial** testing by MLPA
P087 BRCA1 Confirmation	*BRCA1*: all exons	**Confirmation** of MLPA results

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
Fourteen BRCA1 probes have been replaced and two have been added. Probes for *BRCA2* have been removed. Several probes have a change in length but not in sequence detected. The majority of reference probes has been replaced and two additional reference probes have been added.

1. Intended Purpose

The SALSA MLPA Probemix P087 BRCA1 Confirmation 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 *BRCA1* gene in genomic DNA isolated from human peripheral whole blood specimens. P087 BRCA1 Confirmation is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome, as initially determined using the SALSA MLPA Probemix P002 BRCA1. P002 BRCA1 should be used as a first tier probemix, as it provides a more extensive coverage of the *BRCA1* gene.

Discordant results between P087 BRCA1 Confirmation and P002 BRCA1 should be investigated with a different technique.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including 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. • 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.	
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 at MRC Holland	SALSA® Artificial Duplication DNA SD024 (duplication of two probes)
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*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 (*BRCA1*)

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.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions with 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 table above can be used. Cut-off values were verified with P087 BRCA1 Confirmation on 43 samples from healthy individuals with a normal *BRCA1* copy number and six samples with known *BRCA1* copy number variations (CNVs). The expected FRs for the corresponding copy numbers were found in all samples tested.
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 recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P087 BRCA1 Confirmation on two samples with known *BRCA1* CNVs and on one sample with a normal *BRCA1* copy number. The expected results were obtained for all probes and all samples using 250 ng DNA as input amount of DNA. Using 50 ng of DNA, 97.3% of measurements fell within the expected cut-off values. In the remaining 2.7%, final ratios for a sample with a duplication fell within the ambiguous range between the cut-off values for a heterozygous duplication and a heterozygous triplication/homozygous duplication.
Interfering substances	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 P087 BRCA1 Confirmation was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on two samples with known *BRCA1* CNVs and one sample with a normal *BRCA1* copy number. Tested potential interferents, their potential source, testing concentrations and results are presented in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Expected FR for 242/243 measurements
NaCl	Exogenous – DNA extraction	40 mM	Expected FR for 242/243 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Expected FR for 243/243 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Expected FR for 243/243 measurements

	Hemoglobin — Endogenous – blood sample — 0.02 µg/µl — Expected FR for 208/243 measurements Blank (TE) — - — - — Expected FR for 241/243 measurements * Results are summarised for 26 BRCA1 probes and 1 upstream probe across three samples tested in triplicate. $FeCl_3$ and heparin did not interfere with correct copy number determination. EDTA and NaCl led to one out of 243 measurements (<1%) being outside of the expected cut-offs and in the ambiguous range instead, which was comparable to the blank condition. Hemoglobin had a clear effect on final ratios, and even led false negative and false positive results. In the presence of hemoglobin, Coffalyser.Net warnings were obtained. 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.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Experiments on six samples with known *BRCA1* CNVs and one sample with a normal *BRCA1* copy number were carried out using P087 BRCA1 Confirmation to determine whether probes are specific to their target sequence. The expected final ratios were obtained for all probes. Thus, all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, six previously genotyped samples were tested using P087 BRCA1 Confirmation and the expected results were obtained. Assay precision was tested by repeatedly testing two samples with known *BRCA1* CNVs and one sample with a normal *BRCA1* copy number over multiple days, and by multiple operators. Concurrent results were obtained between triplicate reactions. When tested on different days or by different operators, the expected results were obtained in 96.7% and 99.6% of measurements, respectively. In the remaining cases, final ratios of a sample with a duplication fell within the ambiguous range between the cut-off values for a heterozygous duplication and a heterozygous triplication/homozygous duplication in one of the samples tested.
Clinical validity*	80% of hereditary breast and ovarian cancer (HBOC) syndrome cases are linked to *BRCA1* or *BRCA2* mutations. Among these, 66% are due to pathogenic variants in *BRCA1*, of which approximately 11-13% are deletions or duplications that can be detected using gene-targeted deletion/duplication analysis. * Based on a 2000-2023 literature review.

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon MANE Select NM_007294.4	Exon classical numbering	Distance to next probe	Length (nt)	Probe number	Warnings
17q21.31	*BRCA1*	Exon 23	Exon 24	1.9 kb	373	21943-L31019	
17q21.31	*BRCA1*	Exon 22	Exon 23	1.5 kb	418	21947-L30995	
17q21.31	*BRCA1*	Exon 21	Exon 22	1.9 kb	202	11457-L30983	
17q21.31	*BRCA1*	Exon 20	Exon 21	6.1 kb	310	21941-L30756	
17q21.31	*BRCA1*	Exon 19	Exon 20	6.2 kb	391	21945-L30760	
17q21.31	*BRCA1*	Exon 18	Exon 19	0.6 kb	283	21940-L30990	
17q21.31	*BRCA1*	Exon 17	Exon 18	3.8 kb	185	03398-L02254	
17q21.31	*BRCA1*	Exon 16	Exon 17	3.5 kb	409	21946-L30761	
17q21.31	*BRCA1*	Exon 15	Exon 16	3.2 kb	337	02822-L02251	
17q21.31	*BRCA1*	Exon 14	Exon 15	2.1 kb	209	21956-L30984	
17q21.31	*BRCA1*	Exon 13	Exon 14	6.0 kb	454	21949-L30997	
17q21.31	*BRCA1*	Exon 12	Exon 13	0.1 kb	382	21944-L30759	
17q21.31	*BRCA1*	Exon 12	Exon 13	8.4 kb	252	21937-L30986	
17q21.31	*BRCA1*	Exon 11	Exon 12	0.6 kb	290	02819-L30991	
17q21.31	*BRCA1*	Exon 10	Exon 11	1.2 kb	136	21934-L30749	
17q21.31	*BRCA1*	Exon 10	Exon 11	1.9 kb	267	21939-L30988	
17q21.31	*BRCA1*	Exon 10	Exon 11	1.2 kb	447	21948-L30763	
17q21.31	*BRCA1*	Exon 9	Exon 10	1.4 kb	355	03822-L03285	
17q21.31	*BRCA1*	Exon 8	Exon 9	2.5 kb	233	21936-L30751	
17q21.31	*BRCA1*	Exon 7	Exon 8	4.4 kb	155	21935-L30750	
17q21.31	*BRCA1*	Exon 6	Exon 7	0.7 kb	219	02814-L02243	
17q21.31	*BRCA1*	Exon 5	Exon 6	1.6 kb	169	21955-L30982	
17q21.31	*BRCA1*	Exon 4	Exon 5	9.3 kb	347	21942-L30757	
17q21.31	*BRCA1*	Exon 3	Exon 3	8.3 kb	175	02811-L02240	
17q21.31	*BRCA1*	Exon 2	Exon 2	1.4 kb	328	21957-L02239	« #
17q21.31	*BRCA1*	Exon 1	Exon 1a	0.1 kb	436	02100-L30996	« # +
17q21.31	*BRCA1*	Upstream	Upstream (Exon 1a)	0.8 kb	148	02807-L01268	« Ø
17q21.31	*BRCA1*	Upstream	Upstream		259	21938-L30987	« Ø #
2p	Reference				427	08839-L22026	
2q	Reference				274	12782-L30989	
3p	Reference				319	15385-L17792	
5q	Reference				130	00797-L21056	
6p	Reference				160	10686-L30981	
7q	Reference				400	17588-L30994	
9q	Reference				463	08738-L08749	
10p	Reference				226	10244-L30985	
10q	Reference				193	03217-L02642	
11p	Reference				296	18670-L30992	
14q	Reference				364	07034-L30993	
21q	Reference				243	19134-L25333	

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 *BRCA1* exon numbers are derived from the MANE project, and based on the MANE Select transcript. For more information, see the probe sequence document available on the product page on www.mrcholland.com.
The classical exon numbering that lacks an exon 4, from the previous version of this product description is disclosed in a separated column. Annotation of one probe with a target at the edge of or slightly outside the coding region, is altered. The exon numbering of product description version D1-06 is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

«	These probes are 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.
Ø	These probes target sequences outside of the known coding region. Copy number alterations of only (one of) these probes are of unknown clinical significance.
#	The specificity of these probes 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.
+	The ligation sites of this probe is >20 nt away from the nearest exon. For more information, download the probe sequence sheet from the probemix-specific page on www.mrcholland.com.

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. In rare cases, divergent results between P002 BRCA1 and P087 BRCA1 Confirmation can occur if deletion/duplications have breakpoints outside the probe target locations. Discordant results should be investigated with a different technique.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. Deletions of exon 1 and exon 2 are relatively frequent (van den Ouweland et al. 2009), though lower probe signals for these exons should be treated with caution. The presence of salt in the DNA sample can lead to incomplete DNA denaturation, especially of the GC-rich region near exons 1 and 2.
2. Multiple (putative) founder mutations for *BRCA1* have been described, which can cause false positive results.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Del Valle J et al. (2011) Identification of a new complex rearrangement affecting exon 20 of BRCA1. Breast Cancer Res Treat. 130:341-4.
2. van den Ouweland AM et al. (2009). Deletion of exons 1a-2 of BRCA1: a rather frequent pathogenic abnormality. Genet Test Mol Biomarkers. 13:399-406.

Implemented changes in the product description

Version D1-08 – 22 October 2025 (03S)
- Minor textual changes in Intended Purpose and throughout the document.
- Limitation concerning unknown clinical significance of probe 02807-L01268 removed, as this is covered by a probe warning.
- Probemix-specific precaution for divergent results between P002 BRCA1 and P087 BRCA1 Confirmation added.

Version D1-07 – 22 January 2025 (03S)
- Product description adapted to a new template.
- Intended purpose was updated, specifying assay is manual.
- Exon numbering updated to MANE exon numbering.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Flanking probe remark for the BRCA1 upstream probe 21938-L30987 in the Content table was replaced by a new remark to indicate that copy number alterations of only this probe are of unknown clinical significance.
- Warning was added for the 21938-L30987 probe regarding specificity relying on a single nucleotide difference compared to a similar sequence.
- Probemix is now IVDR certified.