Instructions for Use
SALSA® MLPA® Probemix P002 BRCA1

See also the MLPA General Protocol, 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 P002 BRCA1 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P002 BRCA1**
Version	**D1**
Catalogue numbers	**P002-025R (25 reactions)** **P002-050R (50 reactions)** **P002-100R (100 reactions)**
Basic UDI-DI	**872021148P00258**
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 C2 version

Ten additional probes for BRCA1 exons 11, 13, 16 and 24, and two probes for the BRCA1 upstream region have been added. One probe targeting BRCA1 exon 24 and multiple reference probes have been replaced. The hybridising sequence of most probes has been elongated.

1. Intended Purpose

The SALSA MLPA Probemix P002 BRCA1 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 human *BRCA1* gene in genomic DNA isolated from human peripheral whole blood specimens. P002 BRCA1 is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with the P002 BRCA1 probemix should be confirmed with the SALSA MLPA Probemix P087 BRCA1 Confirmation probemix or a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *BRCA1* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix 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. • 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 from MRC Holland	SALSA® Artificial Duplication DNA SD024 (duplication of three 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 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 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 final ratios stated in the table above can be used. Cut-off values were verified with SALSA MLPA Probemix P002 BRCA1 in 153 samples from healthy individuals with a normal *BRCA1* copy number and 20 samples with known *BRCA1* CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Limit of detection	A study that evaluated the acceptable minimum and maximum amount of sample DNA revealed that the use of 50-250 ng of human DNA is the recommended input. 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 P002 BRCA1 on two samples with known *BRCA1* CNVs and on one sample with a normal *BRCA1* copy number. The expected results were obtained using both the lower and upper input amount of DNA.
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 SALSA MLPA Probemix P002 BRCA1 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 319/324 measurements
NaCl	Exogenous – DNA extraction	40 mM	Expected FR for 318/324 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Expected FR for 324/324 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Expected FR for 324/324 measurements

	Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Expected FR for 311/324 measurements
	Blank (TE)	-	-	Expected FR for 324/324 measurements
	* Results are summarised for 36 BRCA1 probes across three samples tested in triplicate. $FeCl_3$ and heparin did not interfere with correct copy number determination. In the presence of EDTA or NaCl, a few measurements were outside the correct cut-off values (1.9% and 2.2%, respectively), and mostly within the ambiguous range. Hemoglobin had the largest 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. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity. Experiments on 20 samples with known *BRCA1* CNVs and one sample with a normal *BRCA1* copy number were carried out using SALSA MLPA Probemix P002 BRCA1 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, nine previously genotyped samples were tested using SALSA MLPA Probemix P002 MLPA and found to have the expected results. 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. Results showed a correct call in 108/108 (between replicates), 324/324 (between days) and 322/324 (between operators) data points, leading to a precision of >99%.			
Clinical validity*	80% of HBOC syndrome cases are linked to *BRCA1* or *BRCA2* aberrations. Among these, 66% are due to pathogenic variant in *BRCA1*, of which approximately 11-13% are deletions/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	Exon classical numbering	Distance to next probe	Length (nt)	Probe number	Warnings
17q21.31	*BRCA1*	Exon 23	Exon 24	0.3 kb	310	20029-L23320	
17q21.31	*BRCA1*	Exon 23	Exon 24	0.1 kb	149	20021-L27332	#
17q21.31	*BRCA1*	Exon 23	Exon 24	1.9 kb	439	18140-L04795	
17q21.31	*BRCA1*	Exon 22	Exon 23	1.5 kb	184	20023-L23035	
17q21.31	*BRCA1*	Exon 21	Exon 22	1.9 kb	412	00785-L23318	
17q21.31	*BRCA1*	Exon 20	Exon 21	6.0 kb	226	20025-L27334	
17q21.31	*BRCA1*	Exon 19	Exon 20	6.3 kb	393	00783-L23319	
17q21.31	*BRCA1*	Exon 18	Exon 19	0.6 kb	214	20024-L23321	
17q21.31	*BRCA1*	Exon 17	Exon 18	3.7 kb	256	20026-L27335	
17q21.31	*BRCA1*	Exon 16	Exon 17	3.3 kb	347	18031-L23028	
17q21.31	*BRCA1*	Exon 15	Exon 16	0.3 kb	196	18144-L22627	
17q21.31	*BRCA1*	Exon 15	Exon 16	3.2 kb	160	20022-L27333	
17q21.31	*BRCA1*	Exon 14	Exon 15	2.1 kb	332	00778-L23026	
17q21.31	*BRCA1*	Exon 13	Exon 14	5.7 kb	269	20027-L27336	
17q21.31	*BRCA1*	Intron 12	Intron 13 (Exon 13)	0.2 kb	459	18169-L23037	Ø
17q21.31	*BRCA1*	Exon 12	Exon 13	0.1 kb	202	18290-L23057	
17q21.31	*BRCA1*	Exon 12	Exon 13	8.5 kb	301	02603-L27340	Δ
17q21.31	*BRCA1*	Exon 11	Exon 12	0.5 kb	358	20031-L23004	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.4 kb	142	18139-L22623	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.5 kb	281	00774-L23003	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.5 kb	427	20036-L27344	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.5 kb	340	20030-L27341	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.4 kb	233	18136-L23325	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.4 kb	296	18135-L27339	
17q21.31	*BRCA1*	Exon 10	Exon 11	0.5 kb	382	20033-L22619	
17q21.31	*BRCA1*	Exon 10	Exon 11	1.1 kb	263	18039-L00345	
17q21.31	*BRCA1*	Exon 9	Exon 10	1.3 kb	251	00772-L23001	
17q21.31	*BRCA1*	Exon 8	Exon 9	2.6 kb	238	01005-L23000	
17q21.31	*BRCA1*	Exon 7	Exon 8	4.3 kb	403	20034-L27629	
17q21.31	*BRCA1*	Exon 6	Exon 7	0.8 kb	220	00769-L22997	
17q21.31	*BRCA1*	Exon 5	Exon 6	1.6 kb	374	20032-L27342	
17q21.31	*BRCA1*	Exon 4	Exon 5	9.3 kb	190	00767-L22995	
17q21.31	*BRCA1*	Exon 3	Exon 3	8.3 kb	421	20035-L22994	
17q21.31	*BRCA1*	Exon 2	Exon 2	1.0 kb	178	00765-L22993	« #
17q21.31	*BRCA1*	Intron 1	Exon 1b	0.2 kb	289	20028-L27338	« ∫ Ø
17q21.31	*BRCA1*	Exon 1	Exon 1a	0.9 kb	154	00763-L22990	« #
17q21.31	*BRCA1*	Upstream	Upstream	3.9 kb	166	02808-L25084	« Ø
17q21.31	*BRCA1*	Upstream	Upstream		324	18142-L23024	« Ø
1p	Reference				275	15112-L27337	
1q	Reference				449	13480-L14942	
2q	Reference				469	09038-L23039	
3q	Reference				208	14684-L03223	
5q	Reference				130	00797-L21056	
6q	Reference				316	07300-L21099	
8q	Reference				366	06760-L24615	
13q	Reference				244	16307-L22396	
15q	Reference				136	17174-L20399	
18q	Reference				172	00808-L00326	

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 sequences document available on the product page at 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. Annotations of one probe with a target at the edge of or slightly outside the coding region was changed. The previous annotation of that probe is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

Δ This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

« 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 289 nt intron 1 probe is located within an alternative first *BRCA1* exon, referred to as exon 1b in the classical exon numbering (Xu et al. 1995). Exon 1b is not present in the MANE Select *BRCA1* transcript NM_007294.4, but the target sequence of the 289 nt probe is included in an extended first exon of other *BRCA1* transcripts (such as NM_001407593.1). The clinical relevance of deletions/duplications of only this probe is unclear.

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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The clinical significance of the following findings is not clear: deletion or duplication of only *BRCA1* intron 1, only the two probes upstream of exon 1, only the intron 12 probe, or only the last two exon 23 probes which are located in the 3'UTR.
2. Deletions of exon 1, intron 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.
3. Multiple (putative) founder mutations for *BRCA1* have been described, which can cause false positive results. These include the c.4964_4982del19 (rs80359876) Southern Italian mutation (Nedelcu et al. 2002) for the 196 nt probe targeting *BRCA1* exon, and the c.5470_5477delATTGGGCA (also known as 5589del8; rs80357973) Chinese mutation (Cao et al. 2016) for the 439 nt probe targeting *BRCA1* exon 23.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Cao WM et al. (2016). Novel germline mutations and unclassified variants of BRCA1 and BRCA2 genes in Chinese women with familial breast/ovarian cancer. BMC Cancer. 16:64.
2. Nedelcu R et al. (2002). BRCA mutations in Italian breast/ovarian cancer families. Eur J Hum Genet. 10:150-2.
3. 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.
4. Xu CF et al. (1995) Distinct transcription start sites generate two forms of *BRCA1* mRNA. Hum Mol Genet.4:2259-64.

Implemented changes in the product description
Version D1-12 – 21 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 two *BRCA1* upstream probes 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. - SNVs rs544342552, rs397509257, and rs138493864 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Probemix is now IVDR certified.