Instructions for Use

SALSA® MLPA® Probemix P077 BRCA2 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 P077 BRCA2 Confirmation product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P077 BRCA2 Confirmation**
Version	**B1**
Catalogue numbers	**P077-025R (25 reactions)** **P077-050R (50 reactions)** **P077-100R (100 reactions)**
Basic UDI-DI	**872021148P07767**
Ingredients	**Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA**

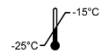
Additional Test Components	**Catalogue numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Artificial Duplication DNA SD024 (optional)	SD024

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 in which the user and/or the patient is located.

Changes in this Product Version

B1 version compared to A3 version

14 target probes and 6 reference probes have been replaced and 2 extra reference probes have been added.

1. Intended Purpose

The SALSA MLPA Probemix P077 BRCA2 Confirmation 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 in genomic DNA isolated from human peripheral whole blood specimens. P077 BRCA2 Confirmation is intended to confirm a potential cause for and clinical diagnosis of hereditary breast and ovarian cancer (HBOC) syndrome, as initially determined using SALSA MLPA Probemix P045 BRCA2/CHEK2 or SALSA MLPA Probemix P090 BRCA2. As they provide a more extensive coverage of the BRCA2 gene, P045 BRCA2/CHEK2 or P090 BRCA2 should be used as a first tier probemix. This P077 BRCA2 Confirmation probemix cannot be used to verify *CHEK2* mutations found with P045 BRCA2/CHEK2. However, the SALSA MLPA Probemix P190 CHEK2 probemix is available for deletion or duplication analysis of other *CHEK2* exons.

Discordant results between the P077 BRCA2 Confirmation probemix and the P045 BRCA2/CHEK2 or P090 BRCA2 probemix 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 2 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 (*BRCA2*)

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

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 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 P077 BRCA2 Confirmation in 66 samples from healthy individuals with a normal *BRCA2* copy number and six samples with known *BRCA2* 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 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 P077 BRCA2 Confirmation on two samples with known *BRCA2* CNVs and on one sample with a normal *BRCA2* copy number and 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 P077 BRCA2 Confirmation was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. For most probes, expected FRs were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 221/222 probes
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 222/222 probes
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 222/222 probes

	<table><tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 222/222 probes</td></tr><tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 189/222 probes</td></tr></table>* Results are summarised for all *BRCA2* probes across all three samples tested in triplicate. $FeCl_3$, NaCl and heparin did not interfere with copy number determination, while one ambiguous result was observed with EDTA. Haemoglobin had the largest effect on the FRs: ambiguous and false results 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. The expected final ratios were obtained from 400/407 (98.28%) data points and no false results were obtained. 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 SALSA MLPA Probemix P077 BRCA2 Confirmation and found to have the expected results. 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 111/111 (between replicates), 333/333 (between days) and 333/333 (between operators) data points, and no false results were obtained, leading to a precision of >99%.
Clinical validity*	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. *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	Distance to next probe	Length (nt)	Probe number	Warnings
13q13.1	*ZAR1L*		1.5 kb	292	08343-L08275	¬
13q13.1	*BRCA2*	Exon 1	0.9 kb	148	20017-L29921	
13q13.1	*BRCA2*	Exon 2	2.6 kb	427	12323-L13316	
13q13.1	*BRCA2*	Exon 3	4.7 kb	256	21499-L29988	
13q13.1	*BRCA2*	Intron 3	1.3 kb	244	12304-L13297	Ø
13q13.1	*BRCA2*	Exon 4	0.9 kb	178	12294-L13287	
13q13.1	*BRCA2*	Exon 5	0.1 kb	337	21503-L29992	
13q13.1	*BRCA2*	Exon 6	0.3 kb	154	21496-L29985	
13q13.1	*BRCA2*	Exon 7	2.0 kb	265	21500-L29989	
13q13.1	*BRCA2*	Intron 7	0.9 kb	202	12297-L13290	Ø
13q13.1	*BRCA2*	Exon 8	0.2 kb	391	21505-L29994	
13q13.1	*BRCA2*	Intron 8 (Exon 8)	1.3 kb	472	12326-L13319	Ø
13q13.1	*BRCA2*	Exon 9	2.0 kb	228	12301-L14436	
13q13.1	*BRCA2*	Exon 10	4.4 kb	355	12315-L13308	
13q13.1	*BRCA2*	Exon 11	2.5 kb	196	12296-L13289	
13q13.1	*BRCA2*	Exon 11	1.3 kb	136	12289-L20891	
13q13.1	*BRCA2*	Exon 11	3.4 kb	160	19614-L26252	
13q13.1	*BRCA2*	Exon 12	0.9 kb	399	21506-L29995	
13q13.1	*BRCA2*	Intron 12	1.4 kb	214	12299-L13292	Ø
13q13.1	*BRCA2*	Exon 13	0.8 kb	328	21502-L29991	
13q13.1	*BRCA2*	Intron 13	7.4 kb	346	12314-L13307	Ø
13q13.1	*BRCA2*	Exon 14	1.4 kb	454	12324-L13317	
13q13.1	*BRCA2*	Exon 15	1.3 kb	418	12322-L13315	
13q13.1	*BRCA2*	Exon 16	0.3 kb	364	12316-L13309	
13q13.1	*BRCA2*	Intron 16 (Exon 16)	4.5 kb	221	12300-L13293	Ø
13q13.1	*BRCA2*	Exon 17	0.8 kb	238	21498-L29987	
13q13.1	*BRCA2*	Exon 18	0.5 kb	463	21508-L29997	
13q13.1	*BRCA2*	Intron 18 (Exon 18)	6.7 kb	274	12307-L13300	Ø
13q13.1	*BRCA2*	Exon 19	0.6 kb	232	12302-L13295	
13q13.1	*BRCA2*	Exon 20	5.7 kb	409	21507-L29996	
13q13.1	*BRCA2*	Exon 21	2.6 kb	301	21501-L29990	
13q13.1	*BRCA2*	Exon 22	0.5 kb	382	21504-L29993	
13q13.1	*BRCA2*	Exon 23	0.2 kb	190	21497-L29986	
13q13.1	*BRCA2*	Exon 24	14.8 kb	436	01618-L14536	
13q13.1	*BRCA2*	Exon 25	2.1 kb	283	12308-L13301	
13q13.1	*BRCA2*	Exon 26	1.4 kb	184	12295-L13288	
13q13.1	*BRCA2*	Exon 27	0.2 kb	166	12292-L14535	
13q13.1	*BRCA2*	Exon 27		310	12310-L13303	
2p	Reference			250	17871-L22467	
2q	Reference			142	14199-L29536	
5q	Reference			130	00797-L13645	
6p	Reference			319	10677-L11259	
6q	Reference			208	13384-L25019	
7q	Reference			124	15370-L13762	
10p	Reference			492	08480-L26254	
11p	Reference			503	06676-L23439	
12q	Reference			373	16494-L18950	
14q	Reference			172	07032-L06643	
15q	Reference			481	09772-L10187	
19p	Reference			444	09077-L23425	

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 based on the MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge or slightly outside the coding region, were altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: Hg18.

7. Precautions and Warnings

Probe warnings

¬ 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.

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.

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 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* 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 B1-10 – 27 October 2025 (03S) - Probemix is now registered for IVD use in Morocco. *Version B1-09 – 17 January 2025 (03S)* - Product description adapted to a new template. - Intended purpose was updated, Fanconi Anemia type D1 removed and specifying assay is manual. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Probemix-specific limitation about the clinical significance of *BRCA2* CNVs in Fanconi Anemia Type D1 was added. - Probemix is now IVDR certified.