Product Description
SALSA® MLPA® Probemix P002-D1 BRCA1

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 12.

Catalogue numbers:
- **P002-025R:** SALSA MLPA Probemix P002 BRCA1, 25 reactions.
- **P002-050R:** SALSA MLPA Probemix P002 BRCA1, 50 reactions.
- **P002-100R:** SALSA MLPA Probemix P002 BRCA1, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.com. It is the responsibility of the user to be aware of the latest scientific knowledge of the application before drawing any conclusions from findings generated with this product.

Intended purpose
The SALSA MLPA Probemix P002 BRCA1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative 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 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 in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

Clinical background
Breast and ovarian carcinomas are among the most common malignancies in developed countries. The majority of cases are considered sporadic, but in a substantial portion, a clear history of cases within a family is present. The BRCA1 and BRCA2 proteins are associated with the activation of double-strand break repair

and homologous recombination and are important in maintaining genomic stability. Germline defects in the *BRCA1* gene are the most frequent cause of a hereditary predisposition to breast cancer. Features characteristic of hereditary, versus sporadic, breast cancer are: younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of diseases such as prostate and breast cancer among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20-25% of hereditary breast cancers (Easton 1999) and about 5-10% of all breast cancers (Campeau et al. 2008). In addition, mutations in the *BRCA1* and *BRCA2* genes account for around 15% of ovarian cancers (Pal et al. 2005). Women with a germline *BRCA1* mutation have a 55-72% risk of developing breast cancer by age 70, while the risk of women in the general population is 12%. The lifetime risk of developing ovarian cancer in women with a germline *BRCA1* mutation is 39-44%, compared to 1-2% in the general population.

The great majority of germline defects in the *BRCA1* gene are point mutations that can be detected by sequence analysis. Deletions and duplications of complete exons in the *BRCA1* gene are the second most common cause of defects in the *BRCA1* gene. These copy number changes are usually missed by amplicon-based sequencing analysis (Sanger sequencing or Next Generation Sequencing), but can be detected by MLPA and hence MLPA complements sequence analysis of the *BRCA1* gene. CNVs in *BRCA1* account for 11-13% of all *BRCA1* pathogenic mutations, dependent on the population. Some populations have a higher prevalence of CNVs in HBOC patients. For example in Italian HBOC families the prevalence is 23% (Montagna et al. 2003), in the Netherlands 27-36% (Hogervorst et al. 2003; Petrij-Bosch et al. 1997), while in a Danish cohort of HBOC patients the prevalence was 3.8% (Thomassen et al. 2006).

More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

Gene structure
The *BRCA1* gene spans ~81 kilobases (kb) on chromosome 17q21.31. The *BRCA1* LRG_292 is available at https://www.lrg-sequence.org/ and is identical to GenBank NG_005905.2. A pseudogene with high sequence similarity to *BRCA1* exons 1a, 1b, and 2 is located 45 kb upstream of *BRCA1* exon 1. All MLPA probes have been designed to detect only the *BRCA1* sequence and not the pseudogene.

Transcript variants
For *BRCA1*, multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/672. Transcript variant 1 is the most predominant and encodes the full-length protein (NM_007294.4, 7088 nt, coding sequence: 114-5705).

Exon numbering
The *BRCA1* exon numbering used in this P002-D1 BRCA1 product description is the traditional exon numbering (exons 1a, 1b, 2, 3 and 5-24), wherein no exon 4 is present. **Please note that the *BRCA1* exon numbering in the *BRCA1* LRG_292 sequence and in the NCBI NG_005905.2 reference sequence is different. In Table 1 and Table 2 the LRG exon numbering is indicated between brackets.** The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P002-D1 BRCA1 contains 48 MLPA probes with amplification products between 130 and 469 nucleotides (nt). This includes 38 probes for the *BRCA1* gene region. At least one MLPA probe is present for each exon in the major *BRCA1* transcript variant 1. Eight probes are present for exon 11 (3426 nt long). Three probes are present for exon 13, which is frequently deleted or duplicated (Hogervorst et al. 2003). Three probes are present for exon 24 and two probes for exon 16. One probe is included for exon 1b, an alternative first exon that is not present in *BRCA1* transcript variant 1, and two probes detect sequences located 4.7 kb and 0.8 kb upstream of the *BRCA1* gene to determine the extent of the deletion or duplication. In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA from human peripheral blood, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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. Reference samples should be derived from different unrelated individuals who are from families without a history of hereditary predisposition to cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA18949, NA14626 and NA14094 from the Coriell Institute have been tested with this P002-D1 probemix at MRC Holland and can be used as positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Coriell Sample ID	Expected result
NA18949	Heterozygous deletion of *BRCA1* exon 15 and 16
NA14626	Heterozygous duplication of *BRCA1* exon 13
NA14094	Heterozygous 40 nt deletion in *BRCA1* exon 11*

* This deletion cannot be detected with the P087 BRCA1 Confirmation probemix.

SALSA Artificial Duplication DNA SD024
In case no positive DNA sample is available in your laboratory, an artificial duplication DNA sample for this probemix (catalogue number SD024) can be ordered from MRC Holland. This SD024 Artificial Duplication DNA

will show a duplication of two or three probes when using the following probemixes: P002 and P087 BRCA1; P045, P090 and P077 BRCA2. The SD024 Artificial Duplication DNA is a mixture of human female genomic DNA and a titrated amount of plasmid containing selected probe target sequences. For further details, please consult the SD024 Artificial Duplication DNA product description, available online: www.mrcholland.com. **This product is for research use only (RUO).**

Performance characteristics
The expected number of *BRCA1* CNVs that can be detected with this MLPA probemix is between 11 and 13% of all *BRCA1* pathogenic mutations, dependent on the population. The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA1* gene in samples is very high and can be considered >99% (based on a 2010-2022 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis
Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results
The expected results for *BRCA1* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication), and occasionally 4 (homozygous duplication or heterozygous triplication, e.g. Hogervorst et al. 2003). A homozygous deletion (copy number 0) of the *BRCA1* gene cannot be expected since such a deletion is associated with embryonic lethality.

The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs,

point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *BRCA1* gene. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.

- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P002 specific notes:

- A hereditary predisposition to breast/ovarian cancer due to *BRCA1* gene defects is an autosomal dominant disorder. Inactivation of a single copy of the *BRCA1* gene is thus expected to be pathogenic.
- A heterozygous deletion of one or more *BRCA1* exons that are present in the major transcript variant NM_007294.4, including the non-coding exon 1a, is expected to result in a hereditary predisposition to breast cancer. The clinical significance of a deletion or duplication of only *BRCA1* exon 1b, which is not present in NM_007294.4, only the exon 13 probe located outside the exon, or only the last two exon 24 probes, which are located in the 3' UTR, is not clear
- Heterozygous deletions of the complete *BRCA1* gene have been described but are rare. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole gene. **Whole gene deletions or duplications should therefore be confirmed by analysis of an *independent* DNA sample, to exclude false positive results.**
- Deletions of exons 1a, 1b and 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 1a, 1b and 2.
- A duplication of an internal part of a gene usually results in a defective copy of that gene, as the duplicated sequence is typically located directly adjacent to the original sequence, resulting in a defective transcript. Duplication of the *complete BRCA1* gene is not expected to be pathogenic, as it does not cause recombination deficiency (Aref-Eshghi et al. 2020).

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *BRCA1* gene are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P002 BRCA1.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) 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.
- Multiple (putative) founder mutations for *BRCA1* have been described, which can cause false positive results (see limitation above). These include the c.4964_4982del19 (rs80359876) Southern Italian mutation (Nedelcu et al. 2002) for the 196 nt probe targeting *BRCA1* exon 16, and the c.5470_5477delATTGGGCA (also known as 5589del8; rs80357973) Chinese mutation (Cao et al. 2016) for the 439 nt probe targeting *BRCA1* exon 24.

Confirmation of results

Copy number changes detected with the P002 BRCA1 probemix should be confirmed. The SALSA MLPA probemix P087 BRCA1 Confirmation can be used for initial confirmation of results. The ligation sites of all probes in the P087 BRCA1 Confirmation probemix have a distance of at least 20 nt from probe ligation sites of the P002 BRCA1 probemix. The SALSA MLPA P239 BRCA1 region probemix can be used to further delineate deletions and duplications that extend outside the *BRCA1* gene. Alternatively, copy number changes can be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

Copy number changes detected by only a single probe always require confirmation with the P087 BRCA1 Confirmation probemix or by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

***BRCA1* mutation database**

https://databases.lovd.nl/shared/genes/BRCA1. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results (e.g., a duplication of *BRCA1* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P002-D1 BRCA1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*BRCA1*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L21056	5q	
136	Reference probe 17174-L20399	15q	
142	**BRCA1 probe** 18139-L22623		**Exon 11 (10)**
149 ◎	**BRCA1 probe** 20021-L27332		**Exon 24 (23)**
154 « ±	**BRCA1 probe** 00763-L22990		**Exon 1a**
160	**BRCA1 probe** 20022-L27333		**Exon 16 (15)**
166 « ¬	Flanking probe 02808-L25084		Upstream
172	Reference probe 00808-L00326	18q	
178 «	**BRCA1 probe** 00765-L22993		**Exon 2**
184	**BRCA1 probe** 20023-L23035		**Exon 23 (22)**
190	**BRCA1 probe** 00767-L22995		**Exon 5 (4)**
196	**BRCA1 probe** 18144-L22627		**Exon 16 (15)**
202	**BRCA1 probe** 18290-L23057		**Exon 13 (12)**
208	Reference probe 14684-L03223	3q	
214	**BRCA1 probe** 20024-L23321		**Exon 19 (18)**
220	**BRCA1 probe** 00769-L22997		**Exon 7 (6)**
226 ±	**BRCA1 probe** 20025-L27334		**Exon 21 (20)**
233	**BRCA1 probe** 18136-L23325		**Exon 11 (10)**
238	**BRCA1 probe** 01005-L23000		**Exon 9 (8)**
244	Reference probe 16307-L22396	13q	
251	**BRCA1 probe** 00772-L23001		**Exon 10 (9)**
256	**BRCA1 probe** 20026-L27335		**Exon 18 (17)**
263	**BRCA1 probe** 18039-L00345		**Exon 11 (10)**
269	**BRCA1 probe** 20027-L27336		**Exon 14 (13)**
275	Reference probe 15112-L27337	1p	
281	**BRCA1 probe** 00774-L23003		**Exon 11 (10)**
289 « ◎ +	**BRCA1 probe** 20028-L27338		**Exon 1b**
296	**BRCA1 probe** 18135-L27339		**Exon 11 (10)**
301 Δ	**BRCA1 probe** 02603-L27340		**Exon 13 (12)**
310 ◎	**BRCA1 probe** 20029-L23320		**Exon 24 (23)**
316	Reference probe 07300-L21099	6q	
324 « ¬	Flanking probe 18142-L23024		Upstream
332	**BRCA1 probe** 00778-L23026		**Exon 15 (14)**
340	**BRCA1 probe** 20030-L27341		**Exon 11 (10)**
347	**BRCA1 probe** 18031-L23028		**Exon 17 (16)**
358	**BRCA1 probe** 20031-L23004		**Exon 12 (11)**
366	Reference probe 06760-L24615	8q	
374	**BRCA1 probe** 20032-L27342		**Exon 6 (5)**
382	**BRCA1 probe** 20033-L22619		**Exon 11 (10)**
393	**BRCA1 probe** 00783-L23319		**Exon 20 (19)**
403	**BRCA1 probe** 20034-L27629		**Exon 8 (7)**
412	**BRCA1 probe** 00785-L23318		**Exon 22 (21)**
421	**BRCA1 probe** 20035-L22994		**Exon 3**
427	**BRCA1 probe** 20036-L27344		**Exon 11 (10)**
439	**BRCA1 probe** 18140-L04795		**Exon 24 (23)**
449	Reference probe 13480-L14942	1q	
459 ◎	**BRCA1 probe** 18169-L23037		**Exon 13 (12)**
469	Reference probe 09038-L23039	2q	

[a] See section Exon numbering on page 2 for more information.

« Probe 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, especially of GC-rich regions.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution and require additional confirmation.

+ This probe is located within *BRCA1* exon 1b (Xu et al. 1995), which is in close proximity to exon 1a of the major *BRCA1* transcript variant 1 (NM_007294.4). The clinical relevance of *BRCA1* exon 1b deletions/duplications is currently unclear.

± SNP rs544342552 (154 nt probe), and SNPs rs397509257 and rs138493864 (226 nt probe) could influence the probe signal. In case of an apparent deletion, it is recommended to sequence the region targeted by the affected probe.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

ɷ The clinical significance of a deletion or duplication of only *BRCA1* exon 1b, only the exon 13 probe located outside the exon, or only the last two exon 24 probes, which are located in the 3' UTR, is not clear.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the table above. Single probe aberration(s) must be confirmed by another method.

Table 2. *BRCA1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*BRCA1* exon[a]	Ligation site NM_007294.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
324 « ¬	18142-L23024	Upstream		TCAGGGTCCTTA-AAATAACAGTCT	3.9 kb
166 « ¬	02808-L25084	Upstream		TCTGCGCACTCG-TAGTTCCACCCC	0.9 kb
154 « # ±	00763-L22990	Exon 1a	73-72, reverse	AGCAGAGGGTGA-AGGCCTCCTGAG	0.2 kb
289 « ɷ +	20028-L27338	Exon 1b	208 nt after Exon 1a	AGGGGGCACTGA-GTGTCCGTGGGG	1.0 kb
		start codon	*114-116 (Exon 2)*		
178 « #	00765-L22993	Exon 2	129-130	ATTTATCTGCTC-TTCGCGTTGAAG	8.3 kb
421	20035-L22994	Exon 3	216-217	TCAAGGAACCTG-TCTCCACAAAGT	9.3 kb
190	00767-L22995	Exon 5 (4)	274-275	ACTTCTCAACCA-GAAGAAAGGGCC	1.6 kb
374	20032-L27342	Exon 6 (5)	354-355	CGAGATTTAGTC-AACTTGTTGAAG	0.8 kb
220	00769-L22997	Exon 7 (6)	518-519	AACCGTGCCAAA-AGACTTCTACAG	4.3 kb
403	20034-L27629	Exon 8 (7)	599-600	CTTGGAACTGTG-AGAACTCTGAGG	2.6 kb
238	01005-L23000	Exon 9 (8)	694-695	CGTTAATAAGGC-AACTTATTGCAG	1.3 kb
251	00772-L23001	Exon 10 (9)	734-735	TTGTTACAAATC-ACCCCTCAAGGA	1.1 kb
263	18039-L00345	Exon 11 (10)	875-876	AAGCGTGCAGCT-GAGAGGCATCCA	0.5 kb
382	20033-L22619	Exon 11 (10)	1329-1330	AGTCTGAATCAA-ATGCCAAAGTAG	0.4 kb
296	18135-L27339	Exon 11 (10)	1719-1718, reverse	CGTTTGGTTAGT-TCCCTGATTTAT	0.4 kb
233	18136-L23325	Exon 11 (10)	2119-2120	CCTACAACTCAT-GGAAGGTAAAGA	0.5 kb
340	20030-L27341	Exon 11 (10)	2629-2630	TGAAGTTAACCA-CAGTCGGGAAAC	0.5 kb
427	20036-L27344	Exon 11 (10)	3146-3147	ATGTCACCTGAA-AGAGAAATGGGA	0.5 kb
281	00774-L23003	Exon 11 (10)	3691-3692	TCCTAGCCCTTT-CACCCATACACA	0.4 kb
142	18139-L22623	Exon 11 (10)	4110-4111	AAAGCCAGGGAG-TTGGTCTGAGTG	0.6 kb
358	20031-L23004	Exon 12 (11)	4258-4259	CTCTGAAGACTG-CTCAGGGCTATC	8.5 kb
301 Δ	02603-L27340	Exon 13 (12)	4354-4355	AATGGCTGAACT-AGAAGCTGTGTT	0.1 kb
202	18290-L23057	Exon 13 (12)	4426-4427	TGACTCTTCTGC-CCTTGAGGACCT	0.2 kb
459 ɷ	18169-L23037	Exon 13 (12)	159 nt after Exon 13	CTCACAACTAAT-ATACCAGTCAGA	5.7 kb
269	20027-L27336	Exon 14 (13)	4529-4530	CCAGAAGGCCTT-TCTGCTGACAAG	2.1 kb
332	00778-L23026	Exon 15 (14)	4663-4664	CTCTGGGAGTCT-TCAGAATAGAAA	3.2 kb
160	20022-L27333	Exon 16 (15)	4819-4820	ATCTGGAATCAG-CCTCTTCTCTGA	0.3 kb
196	18144-L22627	Exon 16 (15)	5096-5097	ACCCCAGAAGAA-TTTGTGAGTGTA	3.3 kb
347	18031-L23028	Exon 17 (16)	5127-5128	TTGCCAGAAAAC-ACCACATCACTT	3.7 kb
256	20026-L27335	Exon 18 (17)	5207-5208	TTTGTGTGTGAA-CGGACACTGAAA	0.6 kb
214	20024-L23321	Exon 19 (18)	5282-5283	ACCCAGTCTATT-AAAGAAAGAAAA	6.3 kb
393	00783-L23319	Exon 20 (19)	5343-5344	TGGTCAATGGAA-GAAACCACCAAG	6.0 kb
226 ±	20025-L27334	Exon 21 (20)	5417-5418	GAAATCTGTTGC-TATGGGCCCTTC	1.9 kb
412	00785-L23318	Exon 22 (21)	5491-5492	TTCTGTGGTGAA-GGAGCTTTCATC	1.5 kb

Length (nt)	SALSA MLPA probe	*BRCA1* exon[a]	Ligation site NM_007294.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
184	20023-L23035	Exon 23 (22)	5535-5536	TCCACCCAATTG-TGGTTGTGCAGC	1.9 kb
439	18140-L04795	Exon 24 (23)	5603-5604	ATGTGTGAGGCA-CCTGTGGTGACC	0.1 kb
		stop codon	*5703-5705 (Exon 24)*		
149 # ◎	20021-L27332	Exon 24 (23)	5717-5718	CTGCAGCCAGCC-ACAGGTACAGAG	0.3 kb
310 ◎	20029-L23320	Exon 24 (23)	6056-6057	GCTGGAAGCACA-GAGTGGCTTGGC	

[a] See section Exon numbering on page 2 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« Probe 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, especially of GC-rich regions.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution and require additional confirmation.

+ This probe is located within *BRCA1* exon 1b (Xu et al. 1995)), which is in close proximity to exon 1a of the major *BRCA1* transcript variant 1 (NM_007294.4). The clinical relevance of *BRCA1* exon 1b deletions/duplications is currently unclear.

This probe's specificity 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.

± SNP rs544342552 (154 nt probe), and SNPs rs397509257 and rs138493864 (226 nt probe) could influence the probe signal. In case of an apparent deletion, it is recommended to sequence the region targeted by the affected probe.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.

◎ The clinical significance of a deletion or duplication of only *BRCA1* exon 1b, only the two, only the exon 13 probe located outside the exon, or only the last two exon 24 probes, which are located in the 3' UTR, is not clear.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Related SALSA MLPA probemixes

P087 BRCA1 Confirmation	Contains probes for the BRCA1 gene. It can be used to confirm the results obtained with probemix P002.
P239 BRCA1 region	Contains probes for the BRCA1 region. It can be used to characterise deletions/duplications extending upstream or downstream of BRCA1. Four probes in probemix P239 have the same ligation sites as probes present in probemix P002.
P045 BRCA2/CHEK2	Contains probes for the BRCA2 and CHEK2 genes, involved in breast and ovarian cancer.
P090 BRCA2	Contains the same probes for the BRCA2 gene as in probemix P045.
P077 BRCA2 Confirmation	Contains probes for the BRCA2 gene. It can be used to confirm the results obtained with probemix P045 or P090.
P190 CHEK2	Contains probes for the CHEK2, ATM and TP53 genes, involved in cancer.
P041/P042 ATM	Contain probes for the ATM gene, involved in breast cancer and Ataxia Telangiectasia.
P056 TP53	Contains probes for TP53, involved in Li-Fraumeni syndrome.
P240 BRIP1/CHEK1	Contains probes for the BRIP1 and CHEK1 genes, involved in breast and ovarian cancer.
P260 PALB2-RAD50-RAD51C-RAD51D	Contains probes for the PALB2, RAD50, RAD51C and RAD51D genes, involved in breast and ovarian cancer.

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P002 product history	
Version	*Modification*
D1	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.
C2	The 88 and 96 nt DNA denaturation control fragments have been replaced (QDX2).
C1	An additional probe for *BRCA1* exon 13 has been added and two reference probes have been replaced.
B1	The probe targeting *BRCA1* exon 24 has been replaced.
A2	The probe targeting *BRCA1* exon 13 has been replaced.
A1	First release.

Implemented changes in the product description

Version D1-11 – 05 April 2023 (04P)
- Expected result with Coriell sample NA14626 corrected to heterozygous duplication of *BRCA1* exon 13.
- Product no longer registered for IVD use in Morocco.

Version D1-10 – 12 January 2023 (04P)
- Coriell sample ID corrected to NA14094.

Version D1-09 – 01 December 2022 (04P)
- Sections Clinical background and Performance characteristics updated with new information.
- Coriell sample added to list of positive samples.
- Clarified that the BRCA1 upstream probes are present to determine the extent of a deletion or duplication in the section Probemix content.
- Clarified that flanking probes are unlikely to have any relation to the condition tested for in the section Interpretation of results.
- In Table 1, the 166 nt and 324 nt probes were denoted as flanking probes and a flanking probe remark warning was added to Table 1 and 2.
- Warning added to Table 1 and 2 for SNPs rs397509257 and rs138493864 which could influence the signal of the 226 nt probe.
- Sections References and Selected publications curated.
- Minor textual and layout changes.

Version D1-08 – 7 June 2021 (04P)
- Intended purpose updated.
- Various minor textual changes.
- Warning added to Table 1 and 2 for SNP rs544342552 which could influence the signal of the 154 nt probe.
- Warning added to Table 1 and 2 regarding the clinical significance of several probes.
- Note below Table 1 and 2 regarding the presence of SNVs updated.
- Section Notes *BRCA1* results updated.
- One reference added and one reference removed from the reference section.

Version D1-07 – 15 February 2021 (04P)
- Product description adapted to a new template.
- Various minor textual changes.
- Transcript variants section updated and incorrect information about the presence of BRCA1 exon 1b in transcript variants 3 and 5 removed.
- Link to *BRCA1* mutation database updated.
- Ligation sites of the probes targeting the *BRCA1* gene updated according to new version of the NM_ reference sequence.
- Clarification about the designation of the *BRCA1* exon 1b probe added to Table 1 and 2.
- Additional information about SALSA MLPA probemix P239 BRCA1 region added to the related probemix section.

- List of selected publications using SALSA MLPA Probemix P002 BRCA1 updated.
- UK has been added to the list of countries in Europe that accept the CE mark.

Version D1-06 – 27 April 2020 (04)
- Product is now registered for IVD use in Costa Rica.

Version D1-05 – 09 January 2019 (04)
- Product is now registered for IVD use in Morocco and Israel.

Version D1-04 – 31 May 2018 (04)
- Product description restructured and adapted to a new template.
- Various minor textual or layout changes.
- Wording of intended use was adjusted.
- Colombia was added as country where product has IVD status.
- Information regarding positive samples (Coriell) was added.
- Information on confirmation of whole gene deletions/duplications was added.
- Limitation on risk of positive results due to founder mutations was added.
- 459 nt probe (18169-L23037) warning under Table 1 and 2 was removed.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- References using P002 were updated.
- Information concerning P087 BRCA1 Confirmation was adjusted (Table 1 and 2 and confirmation of results section) due to an update of the P087 probemix.

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