

# Product Description

## SALSA® MLPA® Probemix P077-B1 BRCA2 Confirmation

To be used with the MLPA General Protocol.

### Version B1

For complete product history see page 10.

### Catalogue numbers:

- **P077-025R:** SALSA MLPA Probemix P077 BRCA2 Confirmation, 25 reactions.
- **P077-050R:** SALSA MLPA Probemix P077 BRCA2 Confirmation, 50 reactions.
- **P077-100R:** SALSA MLPA Probemix P077 BRCA2 Confirmation, 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](http://www.mrcholland.com)).

There are three probemixes available for *BRCA2* testing at MRC Holland. Content and use is described below:

Probemix	Gene 1	Gene 2	Coverage	Use for	Remarks
<b>P045 BRCA2/CHEK2</b>	<i>BRCA2</i>	<i>CHEK2</i>	<i>BRCA2</i> : Each exon <i>CHEK2</i> : Exon 1, 9, 1100delC mutation (exon 11)	Initial testing	Identical <i>BRCA2</i> probes as P090
<b>P090 BRCA2</b>	<i>BRCA2</i>	-	<i>BRCA2</i> : Each exon	Initial testing	Identical <i>BRCA2</i> probes as P045
<b>P077 BRCA2 Confirmation</b>	<i>BRCA2</i>	-	<i>BRCA2</i> : Each exon	Confirmation	<i>BRCA2</i> probes target different ligation sites than probes in P090/P045

### Certificate of Analysis

Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at [www.mrcholland.com](http://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](http://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 P077 BRCA2 Confirmation is an in vitro diagnostic (IVD)<sup>1</sup> or a research use only (RUO) semi-quantitative assay<sup>2</sup> 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, or, in rare cases, Fanconi Anemia type D1, 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 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.

<sup>1</sup> 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).

<sup>2</sup> 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 mutations in the *BRCA1* and *BRCA2* genes are linked to a high risk of young-onset hereditary breast and ovarian cancer. Features characteristic of hereditary, versus sporadic, breast cancer are: younger age at diagnosis, frequent bilateral disease, and more frequent occurrence of disease among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20 to 25% of hereditary breast cancers (Easton 1999) and about 5 to 10% of all breast cancers (Campeau et al. 2008). In addition, mutations in the *BRCA1* and *BRCA2* genes cause around 15% of ovarian cancers overall (Pal et al. 2005).

Deletions or duplications are more frequent for *BRCA1* than for *BRCA2* for most populations. The prevalence of deletions or duplications is dependent on the studied population and ranges from 0% to ~10% of all *BRCA2* mutations (Agata et al. 2005, Woodward et al. 2005, Casilli et al. 2006, Stadler et al. 2010). More information is available at <http://www.ncbi.nlm.nih.gov/books/NBK1247/>.

Biallelic pathogenic variants of *BRCA2* can result in Fanconi Anemia (FA) type D1. FA is characterized by physical abnormalities (such as short stature or abnormal skin pigmentation), bone marrow failure and increased risk for malignancies. The incidence of FA in general is 1:160,000, of which type D1 comprises around 3% of the cases. FA type D1 is associated with early-onset acute leukaemia and solid tumours. More information on FA is available at <https://www.ncbi.nlm.nih.gov/books/NBK1401/>.

### Gene structure

The *BRCA2* gene spans ~84 kilobases (kb) on chromosome 13q13.1 and contains 27 exons. The *BRCA2* LRG\_293 is available at [www.lrg-sequence.org](http://www.lrg-sequence.org) and is identical to GenBank NG\_012772.3.

### Transcript variants

For *BRCA2*, one transcript variant has been described encoding the full length protein (NM\_000059.4; 11954 nt; coding sequence 200-10456; <http://www.ncbi.nlm.nih.gov/gene/675>). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 2 and the stop codon is located in exon 27.

### Exon numbering

The *BRCA2* exon numbering used in this P077-B1 *BRCA2* Confirmation product description is the exon numbering from the LRG\_293 sequence. 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 P077-B1 *BRCA2* Confirmation contains 50 MLPA probes with amplification products between 124 and 503 nucleotides (nt). This includes 38 probes for the *BRCA2* region. At least one MLPA probe is present for each exon in the *BRCA2* transcript. In addition, one probe is included for introns 3, 7, 12 and 13; two probes are included for exons 8, 16, 18 and 27; three probes are included for the large exon 11; and one probe is included detecting a sequence in the upstream region of exon 1. In addition, 12 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](http://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](http://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](http://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  $\leq 0.10$  for all probes over the experiment.

### Required specimens

Extracted DNA from human peripheral whole blood specimens, 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 ( $\geq 3$ ) 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 HBOC syndrome or Fanconi Anemia type D1. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol ([www.mrcholland.com](http://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 NA03330 and NA02718 from the Coriell Institute have been tested with this P077-B1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Coriell Sample ID	Altered target genes in P077-B1	Expected result
NA03330	<i>BRCA2</i>	complete heterozygous <i>BRCA2</i> gene duplication
NA02718	<i>BRCA2</i>	complete heterozygous <i>BRCA2</i> gene deletion

### 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 more probes when using the following probemixes: P077, P045 and P090 *BRCA2*; P002 and P087 *BRCA1*. 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](http://www.mrcholland.com). **This product is for research use only (RUO).**

### Performance characteristics

The frequency of *BRCA2* deletions or duplications in HBOC patients is ~3.4%, dependent on the population (<http://www.ncbi.nlm.nih.gov/books/NBK1247/>). No deletions or duplications for *BRCA2* have been described for FA type D1. The analytical sensitivity and specificity for the detection of deletions or duplications in the *BRCA2* gene in samples without mutations in *BRCA2* is very high and can be considered >99% (based on a 2011-2021 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](http://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 *BRCA2* 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. Judkins et al. 2012). A homozygous deletion (copy number 0) of the *BRCA2* gene is unlikely, but may result in FA type D1 or be embryonically lethal (Loizidou et al. 2016).

The standard deviation of each individual probe over all the reference samples should be  $\leq 0.10$  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. 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 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.

P077 specific notes:

- A hereditary predisposition to breast/ovarian cancer due to *BRCA2* gene defects is an autosomal dominant disorder. Inactivation of a single gene copy of the *BRCA2* gene is thus expected to be pathogenic.
- 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 BRCA2* 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 *BRCA2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P077 *BRCA2* Confirmation.
- 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.
- Several (putative) founder mutations for *BRCA2* have been described, which can cause false positive results (see limitation above).

### Confirmation of results

Detected copy number changes, which are different from those detected with SALSA MLPA Probemix P045 *BRCA2/CHEK2* or SALSA MLPA Probemix P090 *BRCA2* require confirmation 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. Rare cases are known in which results obtained with an

MLPA first tier probemix and a confirmation probemix are different due to a deletion/duplication that has a breakpoint within an exon.

**BRCA2 mutation database**

<https://databases.lovd.nl/shared/genes/BRCA2>. We strongly encourage users to deposit positive results in the LOVD database. 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 *BRCA2* exons 4 and 6 but not exon 5) to MRC Holland: [info@mrcholland.com](mailto:info@mrcholland.com).

**Table 1. SALSA MLPA Probemix P077-B1 BRCA2 Confirmation**

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) <sup>a</sup>	
		Reference	BRCA2
64-105	Control fragments – see table in probemix content section for more information		
124	Reference probe 15370-L13762	7q	
130	Reference probe 00797-L13645	5q	
136	<b>BRCA2 probe</b> 12289-L20891		<b>Exon 11</b>
142	Reference probe 14199-L29536	2q	
148	<b>BRCA2 probe</b> 20017-L29921		<b>Exon 1</b>
154	<b>BRCA2 probe</b> 21496-L29985		<b>Exon 6</b>
160	<b>BRCA2 probe</b> 19614-L26252		<b>Exon 11</b>
166	<b>BRCA2 probe</b> 12292-L14535		<b>Exon 27</b>
172	Reference probe 07032-L06643	14q	
178	<b>BRCA2 probe</b> 12294-L13287		<b>Exon 4</b>
184	<b>BRCA2 probe</b> 12295-L13288		<b>Exon 26</b>
190	<b>BRCA2 probe</b> 21497-L29986		<b>Exon 23</b>
196	<b>BRCA2 probe</b> 12296-L13289		<b>Exon 11</b>
202	<b>BRCA2 probe</b> 12297-L13290		<b>Intron 7</b>
208	Reference probe 13384-L25019	6q	
214	<b>BRCA2 probe</b> 12299-L13292		<b>Intron 12</b>
221	<b>BRCA2 probe</b> 12300-L13293		<b>Exon 16</b>
228	<b>BRCA2 probe</b> 12301-L14436		<b>Exon 9</b>
232	<b>BRCA2 probe</b> 12302-L13295		<b>Exon 19</b>
238	<b>BRCA2 probe</b> 21498-L29987		<b>Exon 17</b>
244	<b>BRCA2 probe</b> 12304-L13297		<b>Intron 3</b>
250	Reference probe 17871-L22467	2p	
256	<b>BRCA2 probe</b> 21499-L29988		<b>Exon 3</b>
265	<b>BRCA2 probe</b> 21500-L29989		<b>Exon 7</b>
274	<b>BRCA2 probe</b> 12307-L13300		<b>Exon 18</b>
283	<b>BRCA2 probe</b> 12308-L13301		<b>Exon 25</b>
292	<b>BRCA2 probe</b> 08343-L08275		<b>Upstream</b>
301	<b>BRCA2 probe</b> 21501-L29990		<b>Exon 21</b>
310	<b>BRCA2 probe</b> 12310-L13303		<b>Exon 27</b>
319	Reference probe 10677-L11259	6p	
328	<b>BRCA2 probe</b> 21502-L29991		<b>Exon 13</b>
337	<b>BRCA2 probe</b> 21503-L29992		<b>Exon 5</b>
346	<b>BRCA2 probe</b> 12314-L13307		<b>Intron 13</b>
355	<b>BRCA2 probe</b> 12315-L13308		<b>Exon 10</b>
364	<b>BRCA2 probe</b> 12316-L13309		<b>Exon 16</b>
373	Reference probe 16494-L18950	12q	
382	<b>BRCA2 probe</b> 21504-L29993		<b>Exon 22</b>
391	<b>BRCA2 probe</b> 21505-L29994		<b>Exon 8</b>
399	<b>BRCA2 probe</b> 21506-L29995		<b>Exon 12</b>
409	<b>BRCA2 probe</b> 21507-L29996		<b>Exon 20</b>
418	<b>BRCA2 probe</b> 12322-L13315		<b>Exon 15</b>
427	<b>BRCA2 probe</b> 12323-L13316		<b>Exon 2</b>
436	<b>BRCA2 probe</b> 01618-L14536		<b>Exon 24</b>
444	Reference probe 09077-L23425	19p	
454	<b>BRCA2 probe</b> 12324-L13317		<b>Exon 14</b>
463	<b>BRCA2 probe</b> 21508-L29997		<b>Exon 18</b>
472	<b>BRCA2 probe</b> 12326-L13319		<b>Exon 8</b>
481	Reference probe 09772-L10187	15q	
492	Reference probe 08480-L26254	10p	
503	Reference probe 06676-L23439	11p	

<sup>a</sup> See section Exon numbering on page 2 for more information.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Detected copy number changes, which are different from those detected with the P045 BRCA2/CHEK2 or P090 BRCA2 probemixes require confirmation by another method.

**Table 2. BRCA2 probes arranged according to chromosomal location**

Length (nt)	SALSA MLPA probe	BRCA2 exon <sup>a</sup>	Ligation site NM_000059.4	Partial sequence <sup>b</sup> (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	200-202 (Exon 2)		
292	08343-L08275	Upstream	1.4 kb before exon 1	ATACTGACCAAT-TTACAGGATTAC	1.5 kb
148	20017-L29921	Exon 1	58-59	GTGTCTTTTGGC-GCGGTGGGTCGC	0.9 kb
427	12323-L13316	Exon 2	206-207	TAAAAATGCCTA-TTGATCCAAAG	2.6 kb
256	21499-L29988	Exon 3	310-311	GAACCTTCTTCA-GAAGCTCCACCC	4.7 kb
244	12304-L13297	Intron 3	1.3 kb before exon 4	TAGTTACCATCA-ACTATTGGAACC	1.3 kb
178	12294-L13287	Exon 4	598-599	GTTTCCTGTCCA-CTTCTAAATTCT	0.9 kb
337	21503-L29992	Exon 5	630-631	TTTTAGTCCTGT-TGTTCTACAATG	0.1 kb
154	21496-L29985	Exon 6	679-680	CCCCCAGTGGTA-TGTGGGAGTTTG	0.3 kb
265	21500-L29989	Exon 7	727-728	GGTCGTCAGACA-CCAAAACATATT	2.0 kb
202	12297-L13290	Intron 7	898 nt before exon 8	CATTGTTTCTCA-AATTGCAGATTA	0.9 kb
391	21505-L29994	Exon 8	836-837	TTACAGTCAGAA-ATGAAGAAGCAT	0.2 kb
472	12326-L13319	Exon 8	143 nt after exon 8	CTGGTCTATAT-GTGATTTAACT	1.3 kb
228	12301-L14436	Exon 9	906-907	TTTTTCCAATCA-TGATGAAAGTCT	2.0 kb
355	12315-L13308	Exon 10	1644-1645	AGACTGCATTCT-TGCAGTAAAGCA	4.4 kb
196	12296-L13289	Exon 11	3197-3198	TCTTAGGTCCAA-TTTCAAATCACA	2.5 kb
136	12289-L20891	Exon 11	5735-5736	CTGCATTTAGGA-TAGCCAGTGGTA	1.3 kb
160	19614-L26252	Exon 11	7038-7039	CCTTATCTTAGT-GGGTAAGTGTTT	3.4 kb
399	21506-L29995	Exon 12	7064-7065	TCAAAGAAACT-TATTAAATGAAT	0.9 kb
214	12299-L13292	Intron 12	770 nt after exon 12	CCCTAAAATGGT-TGTAGATACCTA	1.4 kb
328	21502-L29991	Exon 13	7161-7162	TCGAAGATTGTT-TATGCATCATGT	0.8 kb
346	12314-L13307	Intron 13	788 nt after exon 13	GACCTAGCTCCT-TCCCACACTTGG	7.4 kb
454	12324-L13317	Exon 14	7472-7473	AGTGTGTTAGGA-ATATTAACCTGG	1.4 kb
418	12322-L13315	Exon 15	7701-7702	GAAACAAAGGCA-ACGCGTCTTTCC	1.3 kb
364	12316-L13309	Exon 16	7887-7888	TTTTCAGTTTCA-CACTGAAGATTA	0.3 kb
221	12300-L13293	Exon 16	159 nt after exon 16	TGTAGAAGTCTT-TTGAAAAGTGCT	4.5 kb
238	21498-L29987	Exon 17	8092-8093	ATATGGAACTG-GCAGCTATGGAA	0.8 kb
463	21508-L29997	Exon 18	8364-8365	TATTGAACCTAC-AGATGGGTGGTA	0.5 kb
274	12307-L13300	Exon 18	275 nt after exon 18	TTGCTGCCCTCT-TGTTCTCATAGC	6.7 kb
232	12302-L13295	Exon 19	8623-8624	TTATCATCGCTT-TTCAGTGATGGA	0.6 kb
409	21507-L29996	Exon 20	8775-8776	GGAGGCCCAACA-AAAGAGACTAGA	5.7 kb
301	21501-L29990	Exon 21	8918-8917 reverse	ATTCTTCACTGC-TTCATAAAGCTC	2.6 kb
382	21504-L29993	Exon 22	9010-9009 reverse	TGTTTCTTATCA-TTCAACATTTGC	0.5 kb
190	21497-L29986	Exon 23	9268-9269	TCTGAAAGAGCT-AACATACAGTTA	0.2 kb
436	01618-L14536	Exon 24	9379-9380	TTCAGCAAATTT-TTAGATCCAGAC	14.8 kb
283	12308-L13301	Exon 25	9649-9650	TCTGCTAGTCCA-AAAGAGGGCCAC	2.1 kb
184	12295-L13288	Exon 26	9813-9814	GCCGTACTGC-TCAAATCATTCC	1.4 kb
166	12292-L14535	Exon 27	10083-10084	TCCGGCTGCACA-GAAGGCATTTCA	0.2 kb
310	12310-L13303	Exon 27	10261-10262	GCTCTTTTGTCT-GGTTCAACAGGA	
		<i>stop codon</i>	10454-10456 (Exon 27)		

<sup>a</sup> See section Exon numbering on page 2 for more information.

<sup>b</sup> Only partial probe sequences are shown. Complete probe sequences are available at [www.mrcholland.com](http://www.mrcholland.com). Please notify us of any mistakes: [info@mrcholland.com](mailto:info@mrcholland.com).



SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Detected copy number changes, which are different from those detected with the P045 BRCA2/CHEK2 or P090 BRCA2 probemixes require confirmation by another method.

### Related SALSA MLPA probemixes

P045 BRCA2/CHEK2	Hereditary breast and ovarian cancer syndrome, primary screening <i>BRCA2</i> .
P090 BRCA2	Identical to P045 BRCA2/CHEK2, but does not contain probes for <i>CHEK2</i> .
P002 BRCA1	Hereditary breast and ovarian cancer syndrome, primary screening <i>BRCA1</i> .
P087 BRCA1 Confirmation	Hereditary breast and ovarian cancer syndrome, can be used to confirm the results obtained with P002.
P239 BRCA1 region	Characterisation of deletions/duplications upstream and downstream of <i>BRCA1</i> .
P190 CHEK2	Breast cancer susceptibility, genes included: <i>CHEK2</i> , <i>ATM</i> , <i>TP53</i> .
P056 TP53	Mutations in <i>TP53</i> cause Li-Fraumeni syndrome.
P260 PALB2-RAD50- RAD51C-RAD51D	Probes for the <i>PALB2</i> , <i>RAD51C</i> , <i>RAD51D</i> and <i>RAD50</i> genes, which have been linked to breast and/or ovarian cancer.
P041/P042 ATM	Mutations in <i>ATM</i> have been linked to a higher risk of breast cancer.

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- Gonzalez-Rivera M et al. (2016). Frequency of germline DNA genetic findings in an unselected prospective cohort of triple-negative breast cancer patients participating in a platinum-based neoadjuvant chemotherapy trial. *Breast Cancer Res Treat.* 156:507-515.
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P077 product history	
Version	Modification
B1	14 target probes and 6 reference probes have been replaced and 2 extra reference probes have been added.
A3	The length of the exon 11 probe has been adjusted; no change in hybridising sequence detected.
A2	One reference probe, and the 88 and 96 nt DNA denaturation control fragments (QDX2) have been replaced.
A1	First release.

Implemented changes in the product description
<p>Version B1-06 – 05 July 2021 (04P)</p> <ul style="list-style-type: none"> <li>- Product description rewritten and adapted to a new template.</li> <li>- Intended purpose updated.</li> <li>- UK added to the list of countries in Europe that accept the CE mark.</li> <li>- Transcript variants section, Interpretation of results section, Performance characteristics section and Mutation database section updated.</li> <li>- Ligation sites of the probes targeting the <i>BRCA2</i> gene updated according to new version of the NM_ reference sequence.</li> <li>- References in References section and Selected publications section updated.</li> </ul> <p>Version B1-05 – 25 September 2020 (02P)</p> <ul style="list-style-type: none"> <li>- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.</li> <li>- Product is now registered for IVD use in Costa Rica.</li> </ul> <p>Version B1-04 – 27 April 2020 (02P)</p> <ul style="list-style-type: none"> <li>- Product is now registered for IVD use in Colombia and Israel.</li> </ul> <p>Version B1-03 – 13 May 2019 (02P)</p>

- Product description rewritten and adapted to a new template.
  - Various minor textual or layout changes.
  - Information about Fanconi Anemia type D1 was added to the clinical background and performance characteristics section.
  - Interpretation of results section concerning homozygous deletions (copy number 0) was updated.
  - Limitation on risk of positive results due to founder mutations was added.
  - References which used probemix P077 have been updated.
- Version B1-02 – 11 January 2019 (03)
- Product is now registered for IVD use in Morocco.
- Version B1-01 – 15 September 2017 (03)
- Product description restructured and adapted to a new template.
  - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Version 07 – 19 January 2017 (55)
- Probemix name changed to BRCA2 Confirmation.
  - Table with an overview of BRCA2 probemixes added on page 1.
  - Warning added in Table 1 and Table 2, 148 nt probe 01596-L20889 and 400 nt probe 12320-L13313.
  - New references added on page 2.
  - Minor textual changes throughout the document.

<b>More information:</b> <a href="http://www.mrcholland.com">www.mrcholland.com</a> ; <a href="http://www.mrcholland.eu">www.mrcholland.eu</a>	
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<b>IVD</b>	EUROPE*  COLOMBIA MOROCCO ISRAEL COSTA RICA
<b>RUO</b>	ALL OTHER COUNTRIES

\*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.