# 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).

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

| SALSA MLPA Probemix | Target gene(s) | Coverage | Use for | Remarks |
|---|---|---|---|---|
| **P045 BRCA2/CHEK2** | *BRCA2*, *CHEK2* | *BRCA2:* Each exon<br>*CHEK2:* Exon 1, 9, c.1100delC mutation (exon 11) | Initial testing by MLPA | All BRCA2 probes are identical to those present in P090 BRCA2. |
| **P090 BRCA2** | *BRCA2* | *BRCA2:* Each exon | Initial testing by MLPA | All BRCA2 probes are identical to those present in P045 BRCA2/CHEK2. |
| **P077 BRCA2 Confirmation** | *BRCA2* | *BRCA2:* Each exon | Confirmation of MLPA results | The ligation sites of all P077 BRCA2 probes differ from those targeted by P045/P090 BRCA2. |

**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 P077 BRCA2 Confirmation is an in vitro diagnostic (IVD)[1] or a research use only (RUO) semi-quantitative 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, 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.

[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 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 diseases such as prostate and breast canceramong 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 cause around 15% of ovarian cancers (Pal et al. 2005). Women with a germline *BRCA2* mutation have a 45-62% lifetime risk of developing breast cancer, while the risk of women in the general population is 12%. The lifetime risk of developing ovarian cancer in women with a germline *BRCA2* mutation is 11-17%, compared to 1-2% in the general population. Deletions and duplications are more frequent for *BRCA1* than for *BRCA2* in most populations. CNVs in *BRCA2* account for 2-3% of all pathogenic *BRCA2* mutations, dependent on the population. 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 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, and three probes are included for the large exon 11. One probe is included detecting a sequence upstream of *BRCA2* exon 1 to determine the extent of a deletion or duplication. 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).

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 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 (≥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).

**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 | Expected result |
|---|---|
| NA03330 | Complete heterozygous *BRCA2* gene duplication |
| NA02718 | Complete heterozygous *BRCA2* 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. **This product is for research use only (RUO).**

**Performance characteristics**

The expected number of *BRCA2* CNVs that can be detected with this MLPA probemix is between 2 and 3% of all *BRCA2* pathogenic mutations, dependent on the population. No germline 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 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 *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 ≤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 and 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.

**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, none of which will 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.

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

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

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

¬ 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.

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[a] | Ligation site NM_000059.4 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 292 ¬ | 08343-L08275 | Upstream | | ATACTGACCAAT-TTACAGGATTAC | 1.5 kb |
| | | | | | |
| 148 | 20017-L29921 | Exon 1 | 58-59 | GTGTCTTTTGCG-GCGGTGGGTCGC | 0.9 kb |
| | | *start codon* | *200-202 (Exon 2)* | | |
| 427 | 12323-L13316 | Exon 2 | 206-207 | TAAAAATGCCTA-TTGGATCCAAAG | 2.6 kb |
| 256 | 21499-L29988 | Exon 3 | 310-311 | GAACTTTCTTCA-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 | CTGGTCCTATAT-GTGATTTTAACT | 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-GGGTAAGTGTTC | 3.4 kb |
| 399 | 21506-L29995 | Exon 12 | 7064-7065 | TCAAAAGAAACT-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-ATATTAACTTGG | 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 | ATATGGAAACTG-GCAGCTATGGAA | 0.8 kb |
| 463 | 21508-L29997 | Exon 18 | 8364-8365 | TATTGAACTTAC-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 | GCCGTACACTGC-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 | |
| | | *stop codon* | *10454-10456 (Exon 27)* | | |

[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.

¬ 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.

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 | 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. |
| P190 CHEK2 | Contains probes for the CHEK2, ATM and TP53 genes, involved in cancer. |
| P002 BRCA1 | Contains probes for the BRCA1 gene. It should be used for primary screening of BRCA1. |
| 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. |
| 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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## Selected publications using SALSA MLPA Probemix P077 BRCA2 Confirmation


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- Maistro S et al. (2016). Germline mutations in BRCA1 and BRCA2 in epithelial ovarian cancer patients in Brazil. *BMC Cancer*. 16:934.
- Sahin I et al. (2022). New perspectives on the recurrent BRCA mutations and clinical variability. *Int J Hematol Oncol.* 32:44-9.
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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**

*Version B1-08 – 24 July 2023 (04P)*
- Product is no longer registered as IVD in Morocco.

*Version B1-07 – 01 December 2022 (04P)*
- Table on page 1 updated to clarify that P045/P090 must be used as initial testing by MLPA.
- Sections Clinical background and Performance characteristics updated with new information.
- Clarified that the BRCA2 flanking probe is present to determine the extent of the deletion or duplication in the section Probemix content.
- Clarified that the flanking probe is unlikely to have any relation to the condition tested for in the section Interpretation of results.

- In Table 1, the 292 nt probe was denoted as flanking probe and a flanking probe remark was added in Table 1 and 2.
- Sections References and Selected publications curated.
- Minor textual and layout changes.

*Version B1-06 – 05 July 2021 (04P)*
- Product description rewritten and adapted to a new template.
- Intended purpose updated.
- UK added to the list of countries in Europe that accept the CE mark.
- Transcript variants section, Interpretation of results section, Performance characteristics section and Mutation database section updated.
- Ligation sites of the probes targeting the *BRCA2* gene updated according to new version of the NM_ reference sequence.
- References in References section and Selected publications section updated.

*Version B1-05 – 25 September 2020 (02P)*
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Product is now registered for IVD use in Costa Rica.

*Version B1-04 – 27 April 2020 (02P)*
- Product is now registered for IVD use in Colombia and Israel.

*Version B1-03 — 13 May 2019 (02P)*
- 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.



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|---|---|
| IVD | EUROPE* CE<br>COLOMBIA<br>ISRAEL<br>COSTA RICA |
| RUO | 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.