Product Description SALSA® MLPA® Probemix P002-D1 BRCA1

To be used with the MLPA General Protocol.

Version D1. For a complete product history see page 11.

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, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman capillary sequencers, respectively (see www.mlpa.com).

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

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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 use: This SALSA MLPA probemix P002 BRCA1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) assay for the detection of deletions or duplications in the human *BRCA1* gene in order to confirm a potential cause and clinical diagnosis for hereditary breast and ovarian cancer (HBOC). This product can also be used for molecular genetic testing of at-risk family members.

This assay is for use with human genomic DNA extracted from peripheral blood and not for use with DNA extracted from formalin-fixed paraffin embedded or fresh tumour materials. Deletions or duplications detected with the P002 BRCA1 probemix must be verified by using the SALSA MLPA probemix P087 BRCA1 Confirmation assay or a different technique. In particular, copy number changes detected by only a single probe always require validation 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. This assay is not intended to be used as a standalone assay for clinical decisions. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: 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 disease among male relatives. Mutations in the *BRCA1* and *BRCA2* genes account for about 20 to 25% of hereditary breast cancers and about 5 to 10% of all breast cancers. In addition, mutations in the *BRCA1* and *BRCA2* genes account for around 15% of ovarian cancers overall.
More information is available at http://www.ncbi.nlm.nih.gov/books/NBK1247/.

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 the MLPA technique and hence MLPA complements sequence analysis of the *BRCA1* gene. Large genomic rearrangements (LGRs) in *BRCA1* may account for up to one-third of all disease-causing mutations, dependent on the population (Hansen et al. 2009). 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).

Gene structure: The *BRCA1* gene spans ~80 kilobases (kb) on chromosome 17q21.31. A pseudogene with high sequence similarity to *BRCA1* exons 1a, 1b, and 2 is located 40 kb upstream of exon 1. All MLPA probes have been designed to detect only the *BRCA1* sequence and not the pseudogene. The *BRCA1* LRG_292 is available at www.lrg-sequence.org and is identical to GenBank NG_005905.2.

Transcript variants: Multiple transcript variants have been described: http://www.ncbi.nlm.nih.gov/gene/672. *BRCA1* transcript variant 1 (NM_007294.3, 7224 nt, coding sequence: 233-5824) represents the most abundant transcript, encoding the full-length protein. This sequence is a reference standard in the NCBI RefSeqGene project. The ATG translation start site is located in exon 2 (233-235) and the stop codon is located in exon 24 (5822-5824). *BRCA1* transcript variant 2 (NM_007300.3), variant 3 (NM_007297.3), variant 4 (NM_007298.3), and variant 5 (NM_007299.3) are rare variants that use alternative transcription start sites (exon 1b) and/or alternative in-frame splice sites in the coding sequence. The NM sequence used is from 05/2018, but can be changed (e.g. by NCBI) after the release of the product description.

Exon numbering: The exon numbering used in this P002-D1 BRCA1 product description and in the P002-D1 BRCA1 lot-specific Coffalyser.Net analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. **Please note that the *BRCA1* exon numbering in the *BRCA1* LRG 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.**

Probemix content: This SALSA MLPA probemix P002 BRCA1 contains 48 MLPA probes with amplification products between 130 and 469 nt (Table 1) including 38 probes for the *BRCA1* gene region (Table 2) and 10 reference probes that detect sequences outside this region. The identity of the genes detected by the reference probes is available online (www.mlpa.com).

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, which is the first exon in transcript variants 3 and 5, and two probes detect sequences located 4.6 kb and 0.7 kb upstream of the *BRCA1* gene.

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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (Only visible with <100 ng sample DNA)
88-96	D-fragments (Low signal of 88 or 96 fragment 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.mlpa.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: 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 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.

Positive control DNA samples: Inclusion of a positive sample in each experiment is recommended. Coriell Biobank (https://catalog.coriell.org) and DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID numbers NA18949 and NA14626 from the Coriell Institute have been tested at MRC-Holland and can be used as a positive control sample to detect a deletion of *BRCA1* exons 15 and 16 or a duplication of exon 13, respectively. The quality of cell lines can change, therefore samples should be validated before use.

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.mlpa.com. **This product is for research use only (RUO).**

Performance characteristics: The expected number of *BRCA1* chromosomal rearrangements which can be detected with this MLPA probemix is between ~0.5 and 25% of all *BRCA1* pathogenic mutations, dependent on the population (Smith et al. 2011; Sluiter et al. 2011). The analytical sensitivity and specificity for the detection of deletions/duplications in the *BRCA1* gene in samples without point mutations in *BRCA1* (based on a 2008-2017 literature review), is very high and can be considered >99%.

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) 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 must 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.mlpa.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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results for autosomal or pseudo-autosomal chromosomes:

Copy Number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/ Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. Incomplete DNA denaturation (e.g. due to salt contamination) can 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 (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 in some cases 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.

Notes *BRCA1* results**:**

- 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_007294.3, including the non-coding exon 1a, is expected to result in a hereditary predisposition to breast cancer. The clinical significance of a deletion of only exon 1b, which is not present in NM_007294.3, only the two probes upstream of exon 1, 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 result in disease.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA1* gene are small (point) mutations, most of which will not 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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: Deletions or duplications detected with the P002 BRCA1 probemix must 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, FISH or Southern blotting. For some frequent rearrangements, PCR primers have been described that can be used for confirmation, e.g. exon 13 duplication (*BRCA1*-ins6kbEx13; Puget et al. 1999) and exon 13 deletion (Petrij-Bosch et al. 1997).

Copy number changes detected by only a single probe always require confirmation by the P087 BRCA1 Confirmation probemix or 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: http://research.nhgri.nih.gov/bic/; http://BRCA1.lovd.nl. We strongly encourage users to deposit positive results in the Breast Cancer Mutation Databases. 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 SNPs and unusual results (e.g., a duplication of *BRCA1* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.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	5q31	
136	Reference probe 17174-L20399	15q21	
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 «	**BRCA1 probe** 02808-L25084		**Upstream**
172	Reference probe 00808-L00326	18q21	
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	3q26	
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	13q14	
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	1p33	
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	6q16	
324 «	**BRCA1 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	8q12	
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	1q42	
459	**BRCA1 probe** 18169-L23037		**Exon 13 (12)**
469	Reference probe 09038-L23039	2q37	

(a) Exon numbering used in the P002-D1 BRCA1 product description and in the P002-D1 BRCA1 lot-specific Coffalyser analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. Please note that the *BRCA1* exon numbering in the *BRCA1* LRG sequence and in the NCBI NG_005905.2 reference sequence is different, this exon numbering is indicated between brackets.

« Probe located within, 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 Warning: the exon 13 (12) probe 02603-L27340 can give variable results and needs additional confirmation in case a deletion/duplication of a single probe is found.

Table 2. BRCA1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	BRCA1 exon[a]	Ligation site[b] NM_007294.3	Partial sequence[c] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*233-235 (Exon 2)*		
324 «	18142-L23024	Upstream	4.6 kb upstream exon 1	TCAGGGTCCTTA-AAATAACAGTCT	3.9 kb
166 «	02808-L25084	Upstream	0.7 kb upstream exon 1; reverse	TCTGCGCACTCG-TAGTTCCACCCC	0.9 kb
154 « #	00763-L22990	1a	192-191; reverse	AGCAGAGGGTGA-AGGCCTCCTGAG	0.2 kb
289 «	20028-L27338	1b	exon 1b; 207 nt downstream exon 1a	AGGGGGCACTGA-GTGTCCGTGGGG	1.0 kb
178 « #	00765-L22993	2	248-249	ATTTATCTGCTC-TTCGCGTTGAAG	8.3 kb
421	20035-L22994	3	335-336	TCAAGGAACCTG-TCTCCACAAAGT	9.3 kb
190	00767-L22995	5 (4)	393-394	ACTTCTCAACCA-GAAGAAAGGGCC	1.6 kb
374	20032-L27342	6 (5)	473-474	CGAGATTTAGTC-AACTTGTTGAAG	0.8 kb
220	00769-L22997	7 (6)	637-638	AACCGTGCCAAA-AGACTTCTACAG	4.3 kb
403	20034-L27629	8 (7)	718-719	CTTGGAACTGTG-AGAACTCTGAGG	2.6 kb
238	01005-L23000	9 (8)	813-814	CGTTAATAAGGC-AACTTATTGCAG	1.3 kb
251	00772-L23001	10 (9)	853-854	TTGTTACAAATC-ACCCCTCAAGGA	1.1 kb
263	18039-L00345	11 (10)	994-995	AAGCGTGCAGCT-GAGAGGCATCCA	0.5 kb
382	20033-L22619	11 (10)	1448-1449	AGTCTGAATCAA-ATGCCAAAGTAG	0.4 kb
296	18135-L27339	11 (10)	1838-1837; reverse	CGTTTGGTTAGT-TCCCTGATTTAT	0.4 kb
233	18136-L23325	11 (10)	2238-2239	CCTACAACTCAT-GGAAGGTAAAGA	0.5 kb
340	20030-L27341	11 (10)	2748-2749	TGAAGTTAACCA-CAGTCGGGAAAC	0.5 kb
427	20036-L27344	11 (10)	3265-3266	ATGTCACCTGAA-AGAGAAATGGGA	0.5 kb
281	00774-L23003	11 (10)	3810-3811	TCCTAGCCCTTT-CACCCATACACA	0.4 kb
142	18139-L22623	11 (10)	4229-4230	AAAGCCAGGGAG-TTGGTCTGAGTG	0.6 kb
358	20031-L23004	12 (11)	4377-4378	CTCTGAAGACTG-CTCAGGGCTATC	8.5 kb
301 Δ	02603-L27340	13 (12)	4473-4474	AATGGCTGAACT-AGAAGCTGTGTT	0.1 kb
202	18290-L23057	13 (12)	4545-4546	TGACTCTTCTGC-CCTTGAGGACCT	0.2 kb
459	18169-L23037	13 (12)	159 nt downstream exon 13	CTCACAACTAAT-ATACCAGTCAGA	5.7 kb
269	20027-L27336	14 (13)	4648-4649	CCAGAAGGCCTT-TCTGCTGACAAG	2.1 kb
332	00778-L23026	15 (14)	4782-4783	CTCTGGGAGTCT-TCAGAATAGAAA	3.2 kb
160	20022-L27333	16 (15)	4938-4939	ATCTGGAATCAG-CCTCTTCTCTGA	0.3 kb
196	18144-L22627	16 (15)	5215-5216	ACCCCAGAAGAA-TTTGTGAGTGTA	3.3 kb
347	18031-L23028	17 (16)	5246-5247	TTGCCAGAAAAC-ACCACATCACTT	3.7 kb
256	20026-L27335	18 (17)	5326-5327	TTTGTGTGTGAA-CGGACACTGAAA	0.6 kb
214	20024-L23321	19 (18)	5401-5402	ACCCAGTCTATT-AAAGAAAGAAAA	6.3 kb
393	00783-L23319	20 (19)	5462-5463	TGGTCAATGGAA-GAAACCACCAAG	6.0 kb
226	20025-L27334	21 (20)	5536-5537	GAAATCTGTTGC-TATGGGCCCTTC	1.9 kb
412	00785-L23318	22 (21)	5610-5611	TTCTGTGGTGAA-GGAGCTTTCATC	1.5 kb
184	20023-L23035	23 (22)	5654-5655	TCCACCCAATTG-TGGTTGTGCAGC	1.9 kb
439	18140-L04795	24 (23)	5722-5723	ATGTGTGAGGCA-CCTGTGGTGACC	0.1 kb
149 #	20021-L27332	24 (23)	5836-5837	CTGCAGCCAGCC-ACAGGTACAGAG	0.3 kb
310	20029-L23320	24 (23)	6175-6176	GCTGGAAGCACA-GAGTGGCTTGGC	
		stop codon	*5822-5824 (Exon 24)*		

(a) Exon numbering used in the P002-D1 BRCA1 product description and in the P002-D1 BRCA1 lot-specific Coffalyser analysis sheet is the traditional exon numbering (exons 1a, 2, 3 and 5-24) wherein no exon 4 is present. Please note that the *BRCA1* exon numbering in the *BRCA1* LRG sequence and in the NCBI NG_005905.2 reference sequence is different, this exon numbering is indicated between brackets.

(b) Ligation sites of the P002 BRCA1 MLPA probes are indicated according to RefSeq sequence NM_007294.3 containing 23 exons.

(c) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

« Probe located within, 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 Warning: the exon 13 (12) probe 02603-L27340 can give variable results and needs additional confirmation in case a deletion/duplication of a single probe is found.
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.

Related SALSA MLPA probemixes

▪ P087 BRCA1 Confirmation	Results obtained with P002 can be confirmed with this probemix.
▪ P239 BRCA1 region	Characterisation of *BRCA1* deletions/duplications extending upstream exon 1 or beyond exon 24.
▪ P045 BRCA2/CHEK2	Hereditary breast and ovarian cancer, *BRCA2* and *CHEK2*.
▪ P090 BRCA2	Identical to P045 BRCA2/CHEK2, but does not contain probes for *CHEK2*.
▪ P077 BRCA2 Confirmation	Results obtained with P045 or P090 can be confirmed with this probemix.
▪ P190 CHEK2	Breast cancer susceptibility genes included: *CHEK2*, *ATM*, *TP53*.
▪ P260 PALB2-RAD50-RAD51C-RAD51D	Probes for the *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes, which have been linked to breast and/or ovarian cancer.
▪ P056 TP53	Mutations in *TP53* have been linked to a higher risk of breast cancer.
▪ P041/P042 ATM	Mutations in *ATM* have been linked to a higher risk of breast cancer.
▪ P240 BRIP1/CHEK1	Mutations in *BRIP1* have been linked to a higher risk of ovarian and breast cancer.

References

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P002 Product history	
Version	*Modification*
D1	12 extra *BRCA1* probes and 3 probes for exon 24 have been included, and the reference probes have been replaced. The hybridising sequence of most probes has been elongated.
C2	The 88 and 96 nt DNA denaturation control fragments replaced (QDX2).
C1	A second exon 13 probe is included and 2 reference probes replaced.
B1	The exon 24 probe is replaced.
A2	The exon 13 probe is replaced.
A1	First release.

Implemented changes in the product description

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.

Version D1-03 – 05 May 2017 (03)
- Product description restructured and adapted to a new template.

Version D1-02 – 17 September 2015 (02)
- Table 2: LRG exon numbering corrected for probes 238 nt [exon 9 (8)] and 251 nt [exon 10 (9)]. (Erroneously said, respectively: 238 nt [exon 9 (10]; 251 nt [exon 10 (11)].

Version D1-01 – 07 August 2015 (02)
- Various minor textual changes.
- Warning on overlapping probe sequences with P087 probes adjusted in Table 1 & 2.
- Warning on variability of exon 13 probe 02603-L27340 extended to also include probe 18169-L23037.
- Product description restructured and adapted to a new template.

Version 36 (2)
- Product description restructured.
- Product description adapted to a new product lot (lot number added, changes in Table 1 and Table 2, new picture included).

Version 35 (1)
- Warning added in Table 1 and 2, 160 nt probe 20022-L27333.

Version 34 (1)
- Product description completely rewritten.

- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).

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