Product Description SALSA® MLPA® Probemix P239-C1 BRCA1 region

To be used with the MLPA General Protocol.

Version C1. As compared to version B1, one flanking probe targeting the *STAT3* gene and three reference probes have been replaced. For complete product history see page 6.

Catalogue numbers:
- **P239-025R:** SALSA MLPA Probemix P239 BRCA1 region, 25 reactions.
- **P239-050R:** SALSA MLPA Probemix P239 BRCA1 region, 50 reactions.
- **P239-100R:** SALSA MLPA Probemix P239 BRCA1 region, 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.

General Information: The SALSA MLPA Probemix P239 BRCA1 region is a **research use only (RUO)** assay for the detection of deletions or duplications in the *BRCA1* region, which is associated with hereditary breast cancer.

Defects in the *BRCA1* gene on human chromosome 17q21 are an important cause of hereditary breast cancer. This SALSA® MLPA® P239 BRCA1 region probemix can be used to characterize deletions/duplications that extend to the region up- and downstream of the *BRCA1* gene. For primary screening of *BRCA1*, we recommend using SALSA® MLPA® probemix P002. SALSA® MLPA® probemix P239 BRCA1 region is developed for research purposes to investigate the extent of *BRCA1* deletions/duplications.

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

This SALSA MLPA Probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and Transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

Probemix content: The SALSA MLPA Probemix P239-C1 BRCA1 region contains 32 MLPA probes with amplification products between 130 and 400 nt. This includes 11 probes for the upstream region of *BRCA1* gene including the *NBR1*, *NBR2* genes and the *BRCA1* pseudogene. Furthermore, five probes for the *BRCA1* gene are included and six probes for the downstream region of the *BRCA1* gene including the *VAT1* and *RND2* genes. In addition, ten reference probes are included and detect ten different autosomal chromosomal locations / target relatively quiet regions in the particular type of tumour studied. Complete probe sequences and the identity of the genes detected by the reference probes is available online (www.mlpa.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, 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)

No DNA controls results in only five major peaks shorter than 105 nt: four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 105 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

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

Required specimens: Extracted DNA, which includes DNA derived from paraffin-embedded tissues, 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 healthy individuals without a history of hereditary breast cancer. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive 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. The quality of cell lines can change, therefore samples should be validated before use.

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.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 standard deviation of all probes in the reference samples should be <0.10. 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

Please note that these above mentioned dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- 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. 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 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.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *BRCA1* region are small (point) mutations, most of which will not be detected by using SALSA® MLPA® Probemix P239 BRCA1 region.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample.

Confirmation of results: Copy number changes detected by only a single probe always 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.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

BRCA mutation database: https://www.aruplab.com/topics/breast-cancer/brcadatabase. We strongly encourage users to deposit positive results in the ARUP Laboratories *BRCA1* and *BRCA2* Mutation 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 SNPs and unusual results to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P239-C1 BRCA1 region

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) Reference	*BRCA1* region
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 01212-L00766	8p	
136	**NBR2 probe** 08348-L08280		**Exon 3**
148	**BRCA1P1 probe** 08338-L08270		**Upstream**
157 «	**BRCA1 probe** 00764-L00269		**Exon 1a**
173	**NBR1 probe** 08344-L08276		**Exon 3**
184	Reference probe 04589-L03798	3q	
190	**G6PC probe** 16983-L07227		**Exon 4**
202	Reference probe 13049-L14232	15q	
208	**BRCA1 probe** 00827-L00342		**Exon 6**
214	Reference probe 11396-L12122	1q	
220	**NBR2 probe** 08349-L09138		**Exon 5**
226	**BRCA1P1 probe** 08339-L08271		**Upstream**
232 «	**VAT1 probe** 08335-L19990		**Exon 6**
239 *	Reference probe 21060-L29289	10q	
247	**BRCA1 probe** 16984-L13862		**Exon 24**
256 «	**RND2 probe** 08336-L08268		**Exon 2**
265	**NBR1 probe** 08345-L08277		**Exon 7**
274 «	**GRN probe** 10172-L10625		**Exon 12**
285	**BRCA1 probe** 00775-L00280		**Exon 12**
292	Reference probe 07154-L06766	19q	
301	**NBR1 probe** 16987-L08278		**Exon 11**
310	**NBR2 probe** 16988-L08279		**Exon 1**
318 *	Reference probe 22004-L30847	11q	
328 «	**GRN probe** 10171-L21353		**Exon 10**
337	**BRCA1 probe** 00779-L00003		**Exon 16**
344 *	**STAT3 probe** 16095-L18265		**Exon 7**
352	Reference probe 13681-L15146	5q	
364 «	**RND2 probe** 08337-L08269		**Exon 4**
373 «	**VAT1 probe** 16986-L08266		**Exon 2**
382 *	Reference probe 13329-L14755	18q	
391	**BRCA1P1 probe** 08341-L08273		**Upstream**
400	Reference probe 09109-L09168	4q	

* New in version C1 (from lot C1-0319 onwards).

« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.

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

Length (nt)	SALSA MLPA probe	Gene exon	Ligation site	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
344 *	16095-L18265	STAT3 Exon 7	NM_139276.2; 820-821	GAAACAACCAGT-CAGTGACCAGGC	571.6 kb
190	16983-L07227	G6PC Exon 4	NM_000151.2; 2 nt after Exon 4	TCCTGTCAGGTA-TGGGCTGATCTG	106.7 kb
232 «	08335-L19990	VAT1 Exon 6	NM_006373.4; 1193-1194	GATGCAGGAGAA-GAAGAATGTGGG	2.6 kb
373 «	16986-L08266	VAT1 Exon 2	NM_006373.4; 554-555	CATGACCTTTGA-GGAAGCTGCTGC	7.3 kb
256 «	08336-L08268	RND2 Exon 2	NM_005440.5; 282-283	AAGCGCCGCATT-GAGCTCAACATG	2.1 kb
364 «	08337-L08269	RND2 Exon 4	NM_005440.5; 476-477	GGTTGGCTGTAA-ACTGGACATGCG	17.1 kb
247	16984-L13862	BRCA1 Exon 24	NM_007294.3; 6274-6275	TCAATGGAAGGA-GAGTGCTTGGGA	26.0 kb
337	00779-L00003	BRCA1 Exon 16	NM_007294.3; 4938-4939	ATCTGGAATCAG-CCTCTTCTCTGA	19.8 kb
285	00775-L00280	BRCA1 Exon 12	NM_007294.3; 4377-4378	CTCTGAAGACTG-CTCAGGGCTATC	13.9 kb
208	00827-L00342	BRCA1 Exon 6	NM_007294.3; 473-474	CGAGATTTAGTC-AACTTGTTGAAG	20.1 kb
157 «	00764-L00269	BRCA1 Exon 1a	NM_007294.3; 207 nt after exon 1a	AGGGGGCACTGA-GTGTCCGTGGGG	0.6 kb
310 #	16988-L08279	NBR2 Exon 1	NR_003108.2; 103-104	CGGCTTATTGCA-TCACAGTAATTG	13.1 kb
136 #	08348-L08280	NBR2 Exon 3	NR_003108.2; 340-339 reverse	CACGTGACTACC-TTTTATTGGCAG	6.3 kb
220 #	08349-L09138	NBR2 Exon 5	NR_003108.2; 1307-1308	GAGATCAGCGCA-ATTGTCAGCTAA	23.2 kb
391 #	08341-L08273	BRCA1P1 Upstream	NM_031862.3; 2237 nt before NBR1 reverse	ATTTATCTGCTG-TTTGCGTTGAAG	0.9 kb
226 #	08339-L08271	BRCA1P1 Upstream	NM_031862.3; 1340 nt before NBR1 reverse	GCGGAAGAAGAA-GTGCCAGGAATA	0.5 kb
148 #	08338-L08270	BRCA1P1 Upstream	NM_031862.3; 794 nt before NBR1 reverse	TTTCCGAAGCTA-GGCAGATGGGTA	6.2 kb
173	08344-L08276	NBR1 Exon 3	NM_031862.3; 240-241	ACTTGGGCTGAT-ATCGAAGCTATG	10.5 kb
265	08345-L08277	NBR1 Exon 7	NM_031862.3; 490-491	CACTTGCACATT-ACTCTTCACTGG	5.0 kb
301	16987-L08278	NBR1 Exon 11	NM_031862.3; 1033-1034	AACAGGTTGATA-AGAACTTTCTTA	1187.6 kb
328 «	10171-L21353	GRN Exon 10	NM_002087.2; 1292-1293	GCCAGACCCACA-AGCCTTGAAGAG	0.8 kb
274 «	10172-L10625	GRN Exon 12	NM_002087.2; 1795-1796	GGGAAGGACACT-TCTGCCATGATA	

* New in version C1 (from lot C1-0319 onwards).
« Probe located in or near a GC-rich region. A low signal can be caused by salt contamination in the DNA sample leading to incomplete DNA denaturation, especially of GC-rich regions.
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.

Note:

- For detection of deletions and duplications in the *BRCA1* gene, we recommend SALSA MLPA probemix P002 BRCA1. *BRCA1* deletions and duplications can be confirmed with SALSA MLPA probemix P087 BRCA1.
- The NM_007294.3 sequence represents transcript variant 1 of *BRCA1* and is a reference standard in the NCBI RefSeqGene project. Note: The *BRCA1* exon numbering used in this probemix 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 reference sequence is different.
- The NR_003108.2 sequence represents the non-protein coding RNA sequence of *NBR2*.
- The NM_031862.3 sequence represents transcript variant 3 of *NBR1*.
- For all other genes the mentioned NM_sequence is the reference standard, some of which also used in the NCBI RefSeqGene project.
- The exon numbering and NM sequence used is from 04/2019, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P002 BRCA1: Hereditary breast cancer, primary screening *BRCA1*.
- P087 BRCA1: Hereditary breast cancer, *BRCA1* confirmation.
- P045 BRCA2/CHEK2: Hereditary breast cancer, *BRCA2* and *CHEK2*.
- P090 BRCA2: Identical to P045 BRCA1/CHEK2, but does not contain probes for *CHEK2*.
- P077 BRCA2: Results obtained with P045/P090 can be confirmed with this probemix.
- P190 CHEK2: Breast cancer susceptibility, genes included: *CHEK2*, *ATM*, *BRCA1&2*, *PTEN*, *TP53*.
- P057 FANCD2/PALB2: Mutations in *PALB2* have been linked to a higher risk on breast cancer.
- P240 BRIP1/CHEK1: Mutations in *BRIP1* have been linked to a higher risk on breast cancer.
- P041/P042 ATM: Mutations in *ATM* have been linked to a higher risk on breast cancer.
- P225 PTEN: Mutations in *PTEN* have been linked to a higher risk on breast cancer.

References

- Homig-Holzel C et al. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P239 BRCA1 region

- Peixoto A et al. (2013). Genomic characterization of two large Alu-mediated rearrangements of the BRCA1 gene. *J. Hum. Genet.*, 58, 78.

P239 Product history	
Version	*Modification*
C1	One probe targeting the *STAT3* gene and three reference probes have been replaced.
B1	All targets outside *BRCA1* region have been removed and 5 extra have been added. All reference probes have been replaced, QDX2 fragments added.
A1	First release.

Implemented changes in the product description

Version C1-01 - 25 April 2019 (01P)
- 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).
- Ligation sites of the probes targeting the *VAT1, RND2*, *NBR2* and *NBR1* genes updated according to new version of the NM_reference sequence.
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 11 (55) – 07 March 2016
- Minor textual changes.
- Product description adapted to a new product lot (lot number added, new picture included).

Version 10 (48)
- Electropherogram picture of old buffer (introduced in Dec. 2012) removed.

More information: www.mlpa.com; www.mlpa.eu	
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