# Product Description SALSA® MLPA® Probemix P260-C1 PALB2-RAD50-RAD51C-RAD51D

To be used with the MLPA General Protocol.

**Version C1.** As compared to version B1, two probes for *RAD51C*, two probes for *RAD51D*, one probe for *PALB2* and two reference probes have been replaced. For complete product history see page 10.

**Catalogue numbers:**
- **P260-025R:** SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D, 25 reactions.
- **P260-050R:** SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D, 50 reactions.
- **P260-100R:** SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D, 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:** The SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D is an in vitro diagnostic (IVD)[1] or a research use only (RUO) assay for the detection of deletions or duplications in the human *PALB2*, *RAD51C*, *RAD51D* and *RAD50* genes, in order to determine increased susceptibility to cancer, including but not limited to breast and ovarian cancer. Deletions or duplications in *PALB2* can also confirm a potential cause and clinical diagnosis for autosomal recessive Fanconi-Anemia type N. This product can also be used for molecular genetic testing of at-risk family members.
Of note, all exons of *PALB2, RAD51C* and *RAD51D* are covered, whereas not all exons of *RAD50* are covered in this probemix.

This assay is for use with human DNA extracted from peripheral blood. Deletions or duplications detected with the P260 PALB2-RAD50-RAD51C-RAD51D probemix must be verified by another technique. In particular, deletions or duplications detected by only a single probe always require validation by another method. Most defects in the aforementioned genes are point mutations, none of which are detected by MLPA. It is therefore recommended to use this SALSA MLPA probemix in combination with sequence analysis of these genes. This probemix 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:** *PALB2*, *RAD50*, *RAD51C* and *RAD51D* all play a role in DNA damage repair and a defect in one of these genes can lead to increased risk of tumour formation. For breast cancer, autosomal dominant mutations in the genes *BRCA1* and *BRCA2* are the most frequent cause, followed by mutations in *PALB2*, *CHEK2* and *ATM*, though with a much lower frequency (Buys et al. 2017). Mutations in *PALB2* may also increase the risk of developing pancreatic cancer, although the evidence is limited (see Table 1). When both *PALB2* copies are defect, it can result in Fanconi-Anemia (FA) type N. 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 N comprises less than one percent of the cases. FA type N is associated with an unusually severe predisposition to paediatric malignancies (https://www.ncbi.nlm.nih.gov/books/NBK1401/).

Autosomal dominant mutations in *RAD51C* or *RAD51D* result in increased risk for cancer, in particular ovarian cancer (see Table 1). One article describes a homozygous *RAD51C* point mutation, causing FA-like disorder (FA-type O) (Vaz et al. 2010).
Autosomal dominant *RAD50* mutations are linked to an increased risk to cancer, specifically breast cancer (see Table 1). Compound heterozygosity for two point mutations in *RAD50* has been described, causing Nijmegen breakage syndrome-like disorder, though only one family is mentioned (Waltes et al. 2009).

**Table 1.** Overview of different types of cancer caused by mutations in *PALB2*, *RAD51C*, *RAD51D* and *RAD50*, and its relative risk of developing the cancer.

| Gene | Cancer | Relative risk | Occurrence mutations* | References# |
|---|---|---|---|---|
| **PALB2** | Breast | 33-58% by age 70, with higher risks among those with a greater number of relatives with breast cancer | 1-2% | PMID: 25099575, 17200668, 18628482, 21285249, 26573693 |
| | Pancreatic | Only preliminary evidence | Not known | PMID: 20412113, 19264984, 21285249 |
| **RAD51C** | Ovarian | 5.2-9% | 0.4% to 1.1% | PMID: 26720728, 20400964, 21616938, 22538716, 22451500, 22725699, 26261251, 26689913, 25470109 |
| | Breast | Only preliminary evidence | Not known | PMID: 22725699, 23300655, 25470109, 22451500, 26740214 |
| **RAD51D** | Ovarian | 7-14% | 0.8-1.1% | PMID: 26720728, 23372765, 21822267, 26261251, 22652533, 26718727, 26689913, 27296296 |
| | Breast | Only preliminary evidence | Not known | PMID: 21822267, 23372765, 25445424 |
| **RAD50** | Breast | Only preliminary evidence | Not known | PMID: 24894818, 16474176, 14684699 |

* including point mutations, indels, deletions/duplications. Percentage depends on the population tested, e.g. in *BRCA1/2* mutation negative population percentage can be higher.
# PMID is PubMed unique identifier.

**Gene structure:**
- The *PALB2* gene spans ~38 kilobases (kb) on chromosome 16p12.1 and has 13 exons. The PALB2 LRG_308 is available at www.lrg-sequence.org and is identical to GenBank NG_007406.1.
- The *RAD51C* gene spans ~42 kb on chromosome 17q22 and has 9 exons. The RAD51C LRG_314 is available and identical to GenBank NG_023199.1.
- The *RAD51D* gene spans ~20 kb on chromosome 17q12 and has 10 exons. The RAD51D LRG_516 is available and identical to GenBank NG_031858.1.
- The *RAD50* gene spans ~88 kb on chromosome 5q31.1 and has 25 exons. No LRG is available.

**Transcript variants**:
- For *PALB2* one transcript has been described encoding the full length protein (NM_024675.3; 4069 nt; coding sequence 201-3761; https://www.ncbi.nlm.nih.gov/gene/79728). This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 23.
- *RAD51C* has multiple transcript variants of which transcript variant 1 (NM_058216.1; 1337 nt; coding sequence 43-1173; https://www.ncbi.nlm.nih.gov/gene/5889) is the longest transcript and encodes the longest isoform. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 9.

- *RAD51D* has multiple transcript variants of which transcript variant 1 (NM_002878.3; 2418 nt; coding sequence 257-1243; https://www.ncbi.nlm.nih.gov/gene/5892) encodes isoform 1. This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 10.
- For *RAD50* one transcript has been described encoding the full length protein (NM_005732.3; 6597 nt; coding sequence 402-4340; https://www.ncbi.nlm.nih.gov/gene/10111). This sequence is a reference standard in the RefSeqGene project. The ATG translation start site is located in exon 1 and the stop codon in exon 25.

**Exon numbering:**
- The *PALB2* exon numbering is from the RefSeq transcript NM_024675.3, which is identical to the LRG_308 sequence.
- The *RAD51C* exon numbering is from the RefSeq transcript NM_058216.1, which is identical to the LRG_314 sequence.
- The *RAD51D* exon numbering is from the RefSeq transcript NM_002878.3, which is identical to the LRG_516 sequence.
- The *RAD50* exon numbering is from the RefSeq transcript NM_005732.3.

The exon numbering and NM sequences used are from 03/2018, but can be changed (e.g. by NCBI) after the release of the product description.

**Probemix content:** This SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D contains 50 probes with amplifications products between 130 and 500 nt (Table 2), including 10 reference probes. At least one MLPA probe is present for each exon of *PALB2, RAD51C* and *RAD51D*. For *RAD50* eight probes are divided over the gene, including the first and last exon. 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, 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:** MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. The Coriell Institute (https://catalog.coriell.org) and the Leibniz Institute 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 number NA08039 from the Coriell Institute has been tested at MRC-Holland and can be used as a positive control samples to detect a partial trisomy of chromosome 16p, including a duplication of the complete *PALB2* gene. In addition, NA14230 from the Coriell Institute can be used as a positive control sample to detect a partial chromosome 5 deletion, including a deletion of the complete *RAD50* gene. The quality of cell lines can change, therefore samples should be validated before use.

**Performance characteristics:**
- The frequency of deletions or duplications in *PALB2* in cancer, included but not limited to breast cancer is <1% (Janatova et al. 2013, Tung et al. 2015, Susswein et al. 2016, Blanco et al. 2012), whereas for FA-type N fourteen pathogenic variants in *PALB2* have been reported, of which one was a deletion of exon 1-10 (https://www.ncbi.nlm.nih.gov/books/NBK1401/).
- The frequency of deletions or duplications in *RAD51C* in cancer, included but not limited to ovarian cancer is <1% (Schnurbein et al. 2013, Kraus et al. 2017, Susswein et al. 2016).
- The frequency of deletions or duplications in *RAD51D* in cancer, included but not limited to ovarian cancer is <1% (Susswein et al. 2016, ClinVar at NCBI; gene *RAD51D*).
- The frequency of deletions or duplications in *RAD50* in cancer, included but not limited to breast cancer is <1% (ClinVar at NCBI; gene *RAD50*).

These percentages are dependent on the population tested, e.g. in a population of *BRCA1/2* mutation negative patients, these percentages can be higher.

The analytical sensitivity and specificity for the detection of deletions or duplications in these genes is very high and can be considered >99% (based on a 2013-2017 literature review). 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 *PALB2, RAD51C, RAD51D or RAD50* region specific MLPA probes are allele copy numbers of 2 (normal), 1 (heterozygous deletion), 3 (heterozygous duplication) or 4 (homozygous duplication). A homozygous deletion (copy number 0) of the genes is unlikely to be found in blood derived DNA, because such a deletion is expected to result in 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 |

| Copy Number status | Dosage quotient |
|---|---|
| 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. 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.

**Limitations of the procedure:**
- In most populations, the major cause of genetic defects in the *PALB2, RAD51C, RAD51D and RAD50* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA probemix P260 PALB2-RAD50-RAD51C-RAD51D.
- Not all exons of *RAD50* are covered in this probemix. 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.

**Confirmation of results:** The P057 FANCD2-PALB2 can be used for confirmation of results for seven PALB2 probes of the P260 (see Table 3b). The other six PALB2 probes cannot be confirmed with the P057 probemix because their ligation site is similar or close to the probes in the P260.
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 one or 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.

**Mutation databases:**
For *PALB2*: http://grenada.lumc.nl/LSDB_list/lsdbs/PALB2 can be used.
For *RAD51C*: http://grenada.lumc.nl/LSDB_list/lsdbs/RAD51C can be used.
For *RAD51D*: http://grenada.lumc.nl/LSDB_list/lsdbs/RAD51D can be used.
For *RAD50*: http://grenada.lumc.nl/LSDB_list/lsdbs/RAD50 can be used.

We strongly encourage users to deposit positive results for the different genes in the corresponding 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 (e.g., a duplication of *PALB2* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

## Table 2. SALSA MLPA Probemix P260-C1 PALB2-RAD50-RAD51C-RAD51D

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18) | | | | |
|---|---|---|---|---|---|---|
| | | Reference | *RAD50* | *PALB2* | *RAD51C* | *RAD51D* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | | |
| 130 | Reference probe 19616-L26704 | 4p13 | | | | |
| 136 | **RAD51C probe** 20193-L27481 | | | | **Exon 2** | |
| 142 | **RAD51D probe** 20194-L27482 | | | | | **Exon 10** |
| 148 | **PALB2 probe** 20195-L27483 | | | **Exon 9** | | |
| 154 | **RAD50 probe** 20196-L27484 | | **Exon 14** | | | |
| 160 | Reference probe 09787-L10202 | 15q21 | | | | |
| 166 | **RAD51D probe** 20198-L27486 | | | | | **Exon 6** |
| 172 | **PALB2 probe** 07501-L27938 | | | **Exon 10** | | |
| 178 | **RAD51D probe** 20199-L27487 | | | | | **Exon 1** |
| 184 | **RAD51C probe** 16393-L27657 | | | | **Exon 3** | |
| 190 | Reference probe 08067-L19457 | 9p13 | | | | |
| 196 | **RAD50 probe** 20200-L27488 | | **Exon 25** | | | |
| 202 | **RAD51C probe** 16395-L27659 | | | | **Exon 1** | |
| 208 | **RAD50 probe** 20201-L27489 | | **Exon 10** | | | |
| 217 * | **RAD51D probe** 21961-L30775 | | | | | **Exon 3** |
| 222 * | Reference probe 05709-L15344 | 3q21 | | | | |
| 233 * | **RAD51C probe** 21962-L30776 | | | | **Exon 6** | |
| 238 | **RAD50 probe** 20204-L27492 | | **Exon 23** | | | |
| 245 | **PALB2 probe** 20205-L28341 | | | **Exon 2** | | |
| 253 | **RAD51C probe** 16399-L28036 | | | | **Exon 9** | |
| 262 | Reference probe 16433-L28037 | 18q21 | | | | |
| 267 | **RAD51D probe** 20206-L28038 | | | | | **Exon 9** |
| 274 | **PALB2 probe** 16391-L27658 | | | **Exon 1** | | |
| 281 | **RAD51D probe** 20207-L28039 | | | | | **Exon 7** |
| 288 | **PALB2 probe** 20208-L28040 | | | **Exon 4** | | |
| 293 | Reference probe 15724-L27656 | 12q12 | | | | |
| 301 | **RAD51C probe** 16400-L18815 | | | | **Exon 4** | |
| 310 | **PALB2 probe** 07502-L22722 | | | **Exon 11** | | |
| 319 * | **RAD51D probe** 21963-L30777 | | | | | **Exon 8** |
| 329 * | **RAD51C probe** 21964-L30778 | | | | **Exon 8** | |
| 336 | **RAD50 probe** 08999-L19778 | | **Exon 4** | | | |
| 344 | Reference probe 12785-L27941 | 2q13 | | | | |
| 350 | **PALB2 probe** 07504-L27660 | | | **Exon 13** | | |
| 359 | **RAD51C probe** 20139-L27378 | | | | **Exon 7** | |
| 368 | **RAD51D probe** 20210-L27498 | | | | | **Exon 4** |
| 375 | **PALB2 probe** 07497-L19690 | | | **Exon 6** | | |
| 388 | **RAD51D probe** 20211-L27499 | | | | | **Exon 2** |
| 395 | Reference probe 11901-L27676 | 6p12 | | | | |
| 406 | **PALB2 probe** 20212-L27500 | | | **Exon 3** | | |
| 414 | **RAD50 probe** 20213-L27501 | | **Exon 2** | | | |
| 423 | **PALB2 probe** 20214-L27943 | | | **Exon 8** | | |
| 427 | **RAD51C probe** 20215-L27503 | | | | **Exon 5** | |
| 436 | **RAD50 probe** 09002-L27944 | | **Exon 21** | | | |
| 445 | **RAD50 probe** 08998-L09098 | | **Exon 1** | | | |
| 454 * | Reference probe 15515-L17370 | 7q32 | | | | |
| 463 * | **PALB2 probe** 21965-L30779 | | | **Exon 12** | | |
| 477 | **PALB2 probe** 20217-L27505 | | | **Exon 5** | | |
| 485 | **RAD51D probe** 20218-L27945 | | | | | **Exon 5** |
| 494 | **PALB2 probe** 20219-L27946 | | | **Exon 7** | | |
| 500 | Reference probe 19555-L27674 | 2p13 | | | | |

* New in version C1 (from lot C1-0318 onwards).

## Table 3. P260 probes arranged according to chromosomal location

Table 3a. *RAD50* at 5q31.1

| Length (nt) | SALSA MLPA probe | *RAD50* exon[a] | Ligation site NM_005732.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *402-404 (Exon 1)* | | |
| 445 | 08998-L09098 | Exon 1 | 451-452 | GAGTTTTGGAAT-AGAGGACAAAGA | 1.9 kb |
| 414 | 20213-L27501 | Exon 2 | 572-573 | ACTGGAGATTTC-CCTCCTGGAACC | 20.1 kb |
| 336 | 08999-L19778 | Exon 4 | 818-819 | ATTGACCGAGAA-ATGATCAGTTCT | 11.9 kb |
| 208 | 20201-L27489 | Exon 10 | 1886-1887 | GAGAAAAACAGC-AATGTAGAAACC | 12.2 kb |
| 154 | 20196-L27484 | Exon 14 | 2776-2777 | CCTGACAGATGT-TACAATTATGGA | 14.8 kb |
| 436 | 09002-L27944 | Exon 21 | 3706-3707 | GGATGCTGAGGA-AAAGTATAGAGA | 20.0 kb |
| 238 | 20204-L27492 | Exon 23 | 4012-4011 reverse | CTGCCTTTTGTC-CAGCACTGCATC | 4.1 kb |
| 196 | 20200-L27488 | Exon 25 | 4314-4315 | AATGCAGTGTTA-GCTCCCTGGGAT | |
| | | *stop codon* | *4338-4340 (Exon 25)* | | |

The exon numbering used in this P260-C1 PALB2-RAD50-RAD51C-RAD51D product description for *RAD50* is the exon numbering from the RefSeq transcript NM_005732.3. Ligation sites of the RAD50 MLPA probes are indicated according to RefSeq sequence NM_005732.3 containing 25 exons.

Table 3b. *PALB2* at 16p12.1

| Length (nt) | SALSA MLPA probe | *PALB2* exon[a] | Ligation site NM_024675.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *201-203 (Exon 1)* | | |
| 274 » | 16391-L27658 | Exon 1 | 180-181 | ACGGCTGCTCTT-TTCGTTCTGTCG | 3.1 kb |
| 245 » | 20205-L28341 | Exon 2 | 286-287 | AAGGGAATACAG-CAAGACACTAGC | 0.3 kb |
| 406 | 20212-L27500 | Exon 3 | 5 nt after exon 3 | CACTCAGGTAAA-TCTAGACCATTC | 2.4 kb |
| 288 | 20208-L28040 | Exon 4 | 1343-1344 | CCTAAGAGTCTT-AGCCTGGAAGCA | 5.0 kb |
| 477 | 20217-L27505 | Exon 5 | 1964-1963 reverse | AATGGAGCCGTG-AAAGCATCATCA | 1.2 kb |
| 375 » | 07497-L19690 | Exon 6 | 2741-2742 | CTTCCTGCTTCT-GATAGCATAAAC | 2.9 kb |
| 494 | 20219-L27946 | Exon 7 | 2818-2819 | CGTAGATGTGAG-TGCCATGTTTTG | 2.3 kb |
| 423 | 20214-L27943 | Exon 8 | 2948-2949 | TGTTTTATTTAG-GTTCCAGTATTA | 1.1 kb |
| 148 | 20195-L27483 | Exon 9 | 3170-3171 | CAACAAGTAGAA-GTCATGACGTTT | 1.6 kb |
| 172 » | 07501-L27938 | Exon 10 | 3247-3248 | TATACTAACTTT-TGCTGAGGTCCA | 7.4 kb |
| 310 » | 07502-L22722 | Exon 11 | 3368-3367 reverse | CAGACTGAAGCT-TGGTAAGAATCA | 6.1 kb |
| 463 | 21965-L30779 | Exon 12 | 3487-3486 reverse | TCGTCTTAGGGT-TAATCACAATGA | 4.3 kb |
| 350 » | 07504-L27660 | Exon 13 | 3590-3591 | TGTGCAGCAGCA-ATCTTGACTTCT | |
| | | *stop codon* | *3759-3761 (Exon 13)* | | |

The exon numbering used in this P260-C1 PALB2-RAD50-RAD51C-RAD51D product description for *PALB2* is the exon numbering from the RefSeq transcript NM_024675.3, which is identical to the LRG_308 sequence. Ligation sites of the PALB2 MLPA probes are indicated according to RefSeq sequence NM_024675.3 containing 13 exons.

» The ligation sites of these probes are the same, or in close proximity to probes present in SALSA MLPA P057-B2 probemix.

Table 3c. *RAD51D* at 17q12

| Length (nt) | SALSA MLPA probe | *RAD51D* exon[a] | Ligation site NM_002878.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *257-259 (Exon 1)* | | |
| 178 | 20199-L27487 | Exon 1 | 139-140 | TCTCCTCCGGCA-GCCAGCGCGCCT | 0.6 kb |
| 388 | 20211-L27499 | Exon 2 | 396-395 reverse | AGCTCACCTTGT-AAGACAAGCCAC | 0.5 kb |
| 217 | 21961-L30775 | Exon 3 | 444-443 reverse | TCACGGGGAAAG-CCGAGAACTGAG | 11.2 kb |
| 368 | 20210-L27498 | Exon 4 | 551-552 | CTGGTCTCTATA-CTGGAGAAGTGA | 0.3 kb |
| 485 | 20218-L27945 | Exon 5 | 5 nt before exon 5 | AATGCCCCCACC-CCCAGGTATGTC | 0.7 kb |
| 166 | 20198-L27486 | Exon 6 | 788-789 | ACATCTTCCAGA-TGCTGGATGTGC | 2.9 kb |
| 281 | 20207-L28039 | Exon 7 | 840-841 | TCAGGTGACTGG-TTCTTCAGGAAC | 0.3 kb |
| 319 | 21963-L30777 | Exon 8 | 977-978 | TGGCCCGGGACC-TTGGCATGGCAG | 2.0 kb |
| 267 | 20206-L28038 | Exon 9 | 1060-1061 | GGACGCTCCTGG-AGCTTTGTGCCC | 0.3 kb |

| | | | | | |
|---|---|---|---|---|---|
| 142 | 20194-L27482 | Exon 10 | 1228-1229 | GCCACATTACAG-GGTGATCAGACA | |
| | | *stop codon* | *1241-1243 (Exon 10)* | | |

The exon numbering used in this P260-C1 PALB2-RAD50-RAD51C-RAD51D product description for *RAD51D* is the exon numbering from the RefSeq transcript NM_002878.3, which is identical to the LRG_516 sequence. Ligation sites of the RAD51D MLPA probes are indicated according to RefSeq sequence NM_002878.3 containing 10 exons.

Table 3d. *RAD51C* at 17q22

| Length (nt) | SALSA MLPA probe | *RAD51C* exon[(a)] | Ligation site NM_058216.1 | Partial sequence[(b)] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *43-45 (Exon 1)* | | |
| 202 | 16395-L27659 | Exon 1 | 143-144 | GGGGTTCCAGAC-TGCTGAGGAACT | 2.3 kb |
| 136 | 20193-L27481 | Exon 2 | 340-341 | CCCAGGGCTTCA-TAATCACCTTCT | 1.7 kb |
| 184 | 16393-L27657 | Exon 3 | 578-579 | CTGCATTCAGCA-CCTTCAGCTTAT | 6.5 kb |
| 301 | 16400-L18815 | Exon 4 | 705-706 | GAGTTACTGGCA-CAAGTTTATCTT | 6.7 kb |
| 427 | 20215-L27503 | Exon 5 | 866-867 | CCTTGCAAATAA-TCACAGATTAGC | 10.8 kb |
| 233 | 21962-L30776 | Exon 6 | 936-935 reverse | CCTAATGCAGGA-ACAAGCAAGGCC | 3.3 kb |
| 359 | 20139-L27378 | Exon 7 | 995-996 | CTTTCATTGGGA-CCGAAAGCAAAG | 8.4 kb |
| 329 | 21964-L30778 | Exon 8 | 1032-1031 reverse | TCCTTCTGGCTG-GGTGACTTGTAC | 1.7 kb |
| 253 | 16399-L28036 | Exon 9 | 1122-1123 | TTGCAAACAGAA-GGTTCCTTGAGC | |
| | | *stop codon* | *1171-1173 (Exon 9)* | | |

The exon numbering used in this P260-C1 PALB2-RAD50-RAD51C-RAD51D product description for *RAD51C* is the exon numbering from the RefSeq transcript NM_058216.1, which is identical to the LRG_314 sequence. Ligation sites of the RAD51C MLPA probes are indicated according to RefSeq sequence NM_058216.1 containing 9 exons.

**(a)** The exon numbering and NM sequences used are from 03/2018, but can be changed (e.g. by NCBI) after the release of the product description.

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

## Related SALSA MLPA probemixes

| | |
|---|---|
| P002/P087 BRCA1 | Contain probes for the *BRCA1* gene, involved in breast and ovarian cancer. |
| P045/P090/P077 BRCA2 | Contain probes for the *BRCA2* gene, involved in breast and ovarian cancer. |
| P190 CHEK2 | Contains probes for the genes *CHEK2*, *ATM* and *TP53*, involved in cancer. |
| P057 FANCD2-PALB2 | Contains probes for the *FANCD2* and *PALB2* genes, involved in Fanconi-Anemia. |

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## Selected publications using SALSA® MLPA® Probemix P260 PALB2-RAD50-RAD51C-RAD51D


- Borecka M et al. (2016). Mutation analysis of the PALB2 gene in unselected pancreatic cancer patients in the Czech Republic. *Cancer Genet*. 209:199-204.
- Janatova M et al. (2013). The PALB2 gene is a strong candidate for clinical testing in BRCA1- and BRCA2-negative hereditary breast cancer. *Cancer Epidemiol Biomarkers Prev*. 22:2323-2332.
- Janatova M et al. (2015). Mutation Analysis of the RAD51C and RAD51D Genes in High-Risk Ovarian Cancer Patients and Families from the Czech Republic. *PLoS One*. 10:e0127711.
- Kraus C et al. (2017). Gene panel sequencing in familial breast/ovarian cancer patients identifies multiple novel mutations also in genes others than BRCA1/2. *Int J Cancer*. 140:95-102.
- Poumpouridou N et al. (2016). Development of a novel PTT assay for mutation detection in PALB2 large exons and PALB2 screening in medullary breast cancer. *Fam Cancer*. 15:183-191.
- Schnurbein G et al. (2013). RAD51C deletion screening identifies a recurrent gross deletion in breast cancer and ovarian cancer families. *Breast Cancer Res*. 15:R120.
- Yang C et al. (2016). Characterization of a novel germline PALB2 duplication in a hereditary breast and ovarian cancer family. *Breast Cancer Res Treat*. 160:447-456.


| **P260 Product history** | |
|---|---|
| *Version* | *Modification* |
| C1 | Two probes for *RAD51C*, two probes for *RAD51D*, one probe for *PALB2* and two reference probes have been replaced. |
| B1 | Probes for new gene *RAD51D* have been added, several target probes and reference probes have been replaced/added. |
| A2 | Two reference probes have been replaced and probe for exon 8 of *PALB2* has been adjusted to a 3-part probe. |
| A1 | Probes for new gene *RAD51C* have been added. |
| A | First release. |

**Implemented changes in the product description**

*Version C1-01 – 19 April 2018 (04)*
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 2 and Table 3).
- Warning removed for 07504-L27660 probe.
- Clinical background was rewritten and Table 1 was added.
- A remark was added for probes with ligation sites similar or close to the probes in P057 probemix.

*Version 07 – 29 February 2016 (55)*
- Product description adapted to a new lot (lot number added, new picture included).
- Manufacturers address updated.
- New reference added on page 2.

*Version 06 – 12 February 2015 (54)*
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).

*Version 05 (48)*
- Product description adapted to a new product version (version number changed, lot number added, small

changes in Table 1 and Table 2, new picture included).
*Version 04 (48)*
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

| **More information: www.mlpa.com; www.mlpa.eu** | |
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| | |
|---|---|
| IVD | EUROPE* CE |
| RUO | ALL OTHER COUNTRIES |

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