Instructions for Use

SALSA® MLPA® Probemix P260 PALB2-RAD50-RAD51C-RAD51D

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P260 PALB2-RAD50-RAD51C-RAD51D product page on our website to find Certificates of Analysis and a list of related products.

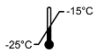
Product Name	**SALSA® MLPA® Probemix P260 PALB2-RAD50-RAD51C-RAD51D**
Version	C1
Catalogue numbers	P260-025R (25 reactions) P260-050R (50 reactions) P260-100R (100 reactions)
Basic UDI-DI	872021148P2605Y
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 ISRAEL
RUO	ALL OTHER COUNTRIES

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version B1, two probes for *RAD51C*, two probes for *RAD51D*, one probe for *PALB2* and two reference probes have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P260 PALB2-RAD50-RAD51C-RAD51D is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *PALB2* and *RAD51C* genes, and deletions in the *RAD51D* gene in genomic DNA isolated from human peripheral whole blood specimens[3]. P260 PALB2-RAD50-RAD51C-RAD51D is intended to confirm a potential cause for and clinical diagnosis of *PALB2*-related cancer predisposition syndrome (*PALB2* deletions/duplications) and *RAD51*-related cancer predisposition syndrome (*RAD51C* deletions/duplications and *RAD51D* deletions). In addition, P260 PALB2-RAD50-RAD51C-RAD51D is intended to establish or confirm a potential cause for and clinical diagnosis of autosomal recessive Fanconi Anemia type N (*PALB2* deletions). This product can also be used for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P260 PALB2-RAD50-RAD51C-RAD51D should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *PALB2, RAD51C* and *RAD51D* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, 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 IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

[3] Probes targeting the *RAD50* gene may be used in a research setting only. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Target
PALB2, RAD51C, RAD51D	*RAD50*

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of predisposition to breast and ovarian cancer, or Fanconi Anemia.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*PALB2, RAD50, RAD51C* and *RAD51D*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations, a study was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. Cut-off values for copy number determination were verified with P260 PALB2-RAD50-RAD51C-RAD51D in five artificial DNA samples mimicking CNVs and normal copy number, 41 DNA samples from healthy individuals with normal copy number and five DNA samples with known CNVs. The expected FRs for the corresponding copy number were found in >99.5% (1,834/1,844) of probe measurements.
Limit of Detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. These lower and higher limits of detection were verified using P260 PALB2-RAD50-RAD51C-RAD51D on a DNA sample with normal copy number and expected FRs were obtained using both the lower and upper input amount of DNA.
Interfering substances	A study using P260 PALB2-RAD50-RAD51C-RAD51D was performed to assess the potential for interference of endogenous and exogenous substances on five artificial DNA samples, four mimicking CNVs and one with normal copy number. Tested potential interferents, their potential source, testing concentrations and results are presented in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 377/420 probe measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 396/420 probe measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 388/420 probe measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 405/420 probe measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 392/420 probe measurements
Blank	-	-	Copy number: Expected FR for 399/420 probe measurements

* Results are summarised for all probes across all five samples tested in triplicate.

Overall, in the blank condition 95% of measurements fell in the expected range. EDTA, haemoglobin and Fe^{3+} had a mild effect on the performance of P260, as slightly more probe measurements fell outside of the expected range than in the blank condition, while heparin and NaCl affected a similar number of

Study	Description
	measurements to the blank condition and therefore had a mild to no impact on probemix performance. All measurements that fell outside of the expected range were in the ambiguous range, and none of the interferents tested had an effect leading to a potential false result.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity. To determine whether probes are specific to their target sequence, five artificial DNA samples mimicking CNVs and normal copy number, 41 blood-derived DNA samples from healthy individuals with normal copy number, and five DNA samples with known CNVs were tested using P260 PALB2-RAD50-RAD51C-RAD51D. The expected FRs for the corresponding copy number were found in >99.5% (1,834/1,844) of measurements across all samples tested.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped DNA samples were tested using P260 PALB2-RAD50-RAD51C-RAD51D and found to have the expected results in >98.4% (248/252) of probe measurements. Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 2,012/2,068 data points, leading to a precision of >97%.
Clinical validity*	*PALB2*- and *RAD51-related cancer predisposition syndromes*: The majority of pathogenic or likely pathogenic CNVs in *PALB2, RAD51C* and *RAD51D* make up an estimated 5-15% of pathogenic variants in the corresponding gene in patients undergoing hereditary cancer predisposition testing (LaDuca et al. 2020). *Fanconi Anemia type N:* Most of the aberrations found in Fanconi Anemia type N patients are point mutations or indels in *PALB2*, however a small number of individuals also have been reported to harbour large deletions (Toksoy et al. 2020, Xia et al. 2007). *Based on a 2013-2026 literature review

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position (chr. pos.)

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
5q31.1	*RAD50*	Exon 1	1.9 kb	445	08998-L09098	
5q31.1	*RAD50*	Exon 2	20.1 kb	414	20213-L27501	
5q31.1	*RAD50*	Exon 4	11.9 kb	336	08999-L19778	
5q31.1	*RAD50*	Exon 10	12.2 kb	208	20201-L27489	
5q31.1	*RAD50*	Exon 14	14.8 kb	154	20196-L27484	
5q31.1	*RAD50*	Exon 21	20.0 kb	436	09002-L27944	
5q31.1	*RAD50*	Exon 23	4.1 kb	238	20204-L27492	
5q31.1	*RAD50*	Exon 25		196	20200-L27488	
16p12.1	*PALB2*	Exon 13	4.3 kb	350	07504-L27660	Δ
16p12.1	*PALB2*	Exon 12	6.1 kb	463	21965-L30779	
16p12.1	*PALB2*	Exon 11	7.4 kb	310	07502-L22722	Δ
16p12.1	*PALB2*	Exon 10	1.6 kb	172	07501-L27938	Δ
16p12.1	*PALB2*	Exon 9	1.1 kb	148	20195-L27483	
16p12.1	*PALB2*	Exon 8	2.3 kb	423	20214-L27943	
16p12.1	*PALB2*	Exon 7	2.9 kb	494	20219-L27946	
16p12.1	*PALB2*	Exon 6	1.2 kb	375	07497-L19690	Δ
16p12.1	*PALB2*	Exon 5	5.0 kb	477	20217-L27505	
16p12.1	*PALB2*	Exon 4	2.4 kb	288	20208-L28040	
16p12.1	*PALB2*	Exon 3	0.3 kb	406	20212-L27500	
16p12.1	*PALB2*	Exon 2	3.1 kb	245	20205-L28341	Δ
16p12.1	*PALB2*	Exon 1		274	16391-L27658	Δ
17q12	*RAD51D*	Exon 10	0.3 kb	142	20194-L27482	
17q12	*RAD51D*	Exon 9	2.0 kb	267	20206-L28038	
17q12	*RAD51D*	Exon 8	0.3 kb	319	21963-L30777	
17q12	*RAD51D*	Exon 7	2.9 kb	281	20207-L28039	
17q12	*RAD51D*	Exon 6	0.7 kb	166	20198-L27486	
17q12	*RAD51D*	Exon 5	0.3 kb	485	20218-L27945	
17q12	*RAD51D*	Exon 4	11.2 kb	368	20210-L27498	
17q12	*RAD51D*	Exon 3	0.5 kb	217	21961-L30775	
17q12	*RAD51D*	Exon 2	0.6 kb	388	20211-L27499	
17q12	*RAD51D*	Exon 1	23.7 **M**b	178	20199-L27487	
17q22	*RAD51C*	Exon 1	2.3 kb	202	16395-L27659	
17q22	*RAD51C*	Exon 2	1.7 kb	136	20193-L27481	
17q22	*RAD51C*	Exon 3	6.5 kb	184	16393-L27657	
17q22	*RAD51C*	Exon 4	6.7 kb	301	16400-L18815	
17q22	*RAD51C*	Exon 5	10.8 kb	427	20215-L27503	
17q22	*RAD51C*	Exon 6	3.3 kb	233	21962-L30776	
17q22	*RAD51C*	Exon 7	8.4 kb	359	20139-L27378	
17q22	*RAD51C*	Exon 8	1.7 kb	329	21964-L30778	
17q22	*RAD51C*	Exon 9		253	16399-L28036	
2p	Reference			500	19555-L27674	
2q	Reference			344	12785-L27941	
3q	Reference			222	05709-L15344	
4p	Reference			130	19616-L26704	
6p	Reference			395	11901-L27676	
7q	Reference			454	15515-L17370	
9p	Reference			190	08067-L19457	
12q	Reference			293	15724-L27656	
15q	Reference			160	09787-L10202	
18q	Reference			262	16433-L28037	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *RAD50, PALB2, RAD51D* and *RAD51C* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

Δ These probes detect the same sequences as probes in SALSA® MLPA® Probemix P057-B3 FANCD2-PALB2.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should

therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. Fanconi Anemia type N is caused by biallelic pathogenic variants in *PALB2,* the majority of which are point mutations and small insertions/deletions that cannot be detected by this product. It is therefore recommended to use this assay in combination with sequence analysis.
6. Somatic reversion has been reported in Fanconi Anemia patients and may result in haemopoietic mosaicism, potentially reducing the detectability of pathogenic variants in peripheral blood samples. In cases where the clinical suspicion of Fanconi Anemia type N is strong but molecular testing of peripheral blood is negative, the possibility of somatic mosaicism should be considered and testing of an alternative cell type may be appropriate.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. Target probes for *RAD50* CNVs are included to be used for research purposes only and not for diagnostic use.
2. This probemix does not include probes for all exons of the *RAD50* gene.
3. Several probes in P260 PALB2-RAD50-RAD51C-RAD51D detect the same sequence as probes in P057-B3 FANCD2-PALB2 (see table Content – Probe Details Sorted by Chromosomal Position). Probes detecting the same sequence cannot be used for result confirmation. Please note that P057 FANCD2-PALB2 is a research use only (RUO) assay and is not intended for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. LaDuca H et al. (2020). A clinical guide to hereditary cancer panel testing: evaluation of gene-specific cancer associations and sensitivity of genetic testing criteria in a cohort of 165,000 high-risk patients. Genet Med. 22:407-415.
2. Toksoy G et al. (2020). Clinical and molecular characterization of Fanconi Anemia patients in Turkey. Mol Syndromol. 11:183-196.
3. Xia B et al. (2007). Fanconi anemia is associated with a defect in the BRCA2 partner PALB2. Nat Genet. 39:159-161.

Implemented changes in the product description

Version C1-06 – 29 July 2026 (03S)
- Product description was updated to new template.
- Intended purpose updated, specifying the assay is manual, removing the *RAD50* gene for diagnostic use, limiting aberrations in *RAD51D* to deletions, changing disease nomenclature to *PALB2*-related cancer predisposition syndrome and *RAD51*-related cancer predisposition syndrome, removing the specification that the device should only be used on those who are *BRCA1* and *BRCA2* mutation negative, and specifying which genetic aberration corresponds to each disease.
- Precaution added regarding the types of pathogenic variants which cause Fanconi Anemia type N.
- Precaution added regarding somatic reversion in Fanconi Anemia.
- Limitation regarding confirmation with P057-B3 FANCD2-PALB2 reworded.
- Limitation added that probes for *RAD50* can only be used in a research setting.
- Probemix is now IVDR certified.

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