Instructions for Use
SALSA® MLPA® Probemix P226 SDH

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 P226 SDH product page on our website to find Certificates of Analysis and a list of related products.

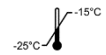
Product Name	**SALSA® MLPA® Probemix P226 SDH**
Version	D1
Catalogue numbers	P226-025R (25 reactions) P226-050R (50 reactions) P226-100R (100 reactions)
Basic UDI-DI	872021148P2265Y
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA 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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E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
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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 in which the user and/or the patient is located.

Changes in this Product Version

As compared to version C1, one target probe for *SDHB* exon 1, one target probe for *SDHC* exon 4 and one target probe for *SDHAF1* exon 1 were replaced. Additional target probes for *SDHC* exon 3, *SDHC* exon 6, *SDHD* exon 3 and *SDHD* exon 4 were included. Three reference probes were replaced and one reference probe was added. Two probes have a small change in length but no change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P226 SDH 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 *SDHB* and deletions in *SDHC* and *SDHD* in genomic DNA isolated from human peripheral whole blood specimens. P226 SDH is intended to confirm a potential cause for and clinical diagnosis of Hereditary Paraganglioma/Pheochromocytoma (PGL/PCC) and for molecular genetic testing of at-risk family members[3].

Copy number variations (CNVs) detected with P226 SDH 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 *SDHB*, *SDHC* and *SDHD* genes are point mutations, none of which will be detected by P226 SDH. 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] Certain probes targeting additional genes included in P226 SDH may only be used in a research setting. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Targets
SDHB, SDHC, SDHD	*SDHAF1, SDHAF2*

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, 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 is 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 PGL/PCC.	
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 (*SDHB*, *SDHC*, *SDHD*, *SDHAF1*, *SDHAF2*)

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

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy numbers in normal and affected populations</td><td>To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in section 5 above can be used. Cut-off values for copy number determination were verified with P226 SDH in 86 samples from healthy individuals with normal copy numbers, five cell-line derived samples with known CNVs and five control plasmid samples with known CNVs. Some deviations were observed in the control plasmid DNA., and one deviating measurement was observed in a cell-line derived sample. FRs corresponding to the expected copy number were still obtained in in 99.2% (2250/2266) of measurements.</td></tr>
<tr><td>Limit of detection</td><td>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. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using P226 SDH on one sample with no aberrations, and expected results were obtained using both the lower and upper input amount of DNA. The lower DNA input was also verified using five samples with known CNVs and only one deviating probe measurement was observed.</td></tr>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using P226 SDH was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on control plasmid samples with known CNVs. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Expected FR for 320/330 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 330/330 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 329/330 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 327/330 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 220/330 measurements</td></tr>
</table>
* Results are summarised for all probes across all five samples tested.

NaCl did not interfere with copy number determination, while an effect on the final ratios (FRs) was observed for a low number of probes with EDTA, $FeCl_3$, and heparin. Hemoglobin had the largest effect on the FRs.

To minimise variability across 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.</td></tr>
<tr><td>Cross-reactivity</td><td>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.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using P226 SDH and found to have the expected results, with the exception of a single measurement. 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 1634/1650 data points, leading to a precision of 99%.</td></tr>
<tr><td>Clinical validity*</td><td>SDHB: 2.5-8.25% of hereditary PGL/PCC is caused by deletions or duplications in this gene (GeneReviews)
SDHC: ~1.2% of hereditary PGL/PCC is caused by deletions in this gene (GeneReviews)
SDHD: ~1-2.5% of hereditary PGL/PCC is caused by deletions in this gene (GeneReviews)
*(Based on a 2009-2025 literature review)</td></tr>
</table>

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

Chromosomal position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
1p36.13	*SDHB*	Exon 8	3.8 kb	310	15741-L06981	
1p36.13	*SDHB*	Exon 7	1.3 kb	427	16976-L19974	
1p36.13	*SDHB*	Exon 6	3.8 kb	238	07347-L06979	
1p36.13	*SDHB*	Exon 5	0.9 kb	483	16980-L19978	
1p36.13	*SDHB*	Exon 4	4.4 kb	373	14872-L16797	
1p36.13	*SDHB*	Exon 3	11.7 kb	211	11094-L30475	
1p36.13	*SDHB*	Exon 2	9.3 kb	232	16967-L19965	
1p36.13	*SDHB*	Exon 1	0.1 kb	463	16978-L19976	+
1p36.13	*SDHB*	Upstream	142.3 **M**b	202	21768-L30666	Ø
1q23.3	*SDHC*	Upstream	0.6 kb	142	07350-L16209	Ø
1q23.3	*SDHC*	Intron 1	9.1 kb	190	16964-L19962	Ø
1q23.3	*SDHC*	Exon 2	4.8 kb	364	16974-L19972	
1q23.3	*SDHC*	Exon 3	0.2 kb	384	14642-L16292	
1q23.3	*SDHC*	Exon 3	12.1 kb	270	14641-L16291	+
1q23.3	*SDHC*	Exon 4	0.1 kb	286	21559-L30106	
1q23.3	*SDHC*	Intron 4	16.1 kb	445	16977-L19975	Ø
1q23.3	*SDHC*	Exon 5	5.5 kb	155	16961-L19959	
1q23.3	*SDHC*	Exon 6	1.0 kb	250	07356-L30156	
1q23.3	*SDHC*	Downstream		319	16972-L19970	Ø
11q12.2	*SDHAF2*	Exon 1	7.5 kb	160	14639-L16289	
11q12.2	*SDHAF2*	Exon 2	0.3 kb	196	16965-L19963	
11q12.2	*SDHAF2*	Exon 3	8.0 kb	393	14643-L21022	
11q12.2	*SDHAF2*	Exon 4	50.5 **M**b	418	14646-L16296	
11q23.1	*SDHD*	Upstream	0.3 kb	326	07357-L16211	Ø
11q23.1	*SDHD*	Exon 1	1.1 kb	292	16971-L19969	Δ
11q23.1	*SDHD*	Exon 2	0.9 kb	355	16973-L19971	
11q23.1	*SDHD*	Exon 3	0.2 kb	264	21558-L30105	
11q23.1	*SDHD*	Exon 3	5.8 kb	172	16962-L19960	+
11q23.1	*SDHD*	Exon 4	0.5 kb	244	21557-L30298	
11q23.1	*SDHD*	Exon 4		220	07361-L20367	#
19q13.12	*SDHAF1*	Exon 1	0.3 kb	166	21556-L30299	
19q13.12	*SDHAF1*	Exon 1		136	14638-L16288	
3p	Reference			336	05433-L04849	
5q	Reference			130	00797-L00463	
6q	Reference			472	13413-L14870	
7q	Reference			400	07991-L07772	
7q	Reference			178	02958-L02390	
8q	Reference			454	08274-L08153	
12q	Reference			303	05697-L05139	
14q	Reference			279	12437-L13438	
17q	Reference			148	08578-L08579	
18q	Reference			436	13340-L14766	
20p	Reference			256	20618-L30934	
21q	Reference			494	19137-L27130	
22q	Reference			226	12269-L13212	

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 *SDHB, SDHC, SDHD, SDHAF1, and SDHAF2* exon numbers are derived from the MANE project and are based on MANE Select transcripts. 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	This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.
Ø	These probes target sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.
#	The specificity of this probe 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.
+	The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.

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 for.
5. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. Target probes for *SDHAF1* and *SDHAF2* CNVs are included to be used for research purposes only and not for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Bayley JP et al. (2005). The SDH mutation database: an online resource for succinate dehydrogenase sequence variants involved in pheochromocytoma, paraganglioma and mitochondrial complex II deficiency. BMC Med Genet. 6:39.
2. Bayley JP et al. (2009). The first Dutch SDHB founder deletion in paraganglioma-pheochromocytoma patients. BMC Med Genet. 10:34.
3. Buffet A et al. (2012). A decade (2001-2010) of genetic testing for pheochromocytoma and paraganglioma. Horm Metab Res. 44:359-366Article 3

Implemented changes in the product description
Version D1-10 – 23 March 2026 (03S) - Intended purpose updated: assay now described as semi-quantitative *manual* assay; *SDHAF1* and *SDHAF2* removed due to lack of scientific validity; specification added that for *SDHC* and *SDHD*, only deletions are associated with the syndrome; a footnote has been added for the distinction between RUO and IVD targets. - Basic UDI-DI added. - Notified body number added to CE certification symbol. - Reference to the SSP added. - Performance characteristics updated. - Probemix-specific limitation related to the RUO status of SDHAF1 and SDHAF2 probes added. - Exon numbering from version D1-08 of this document (present between brackets in version D1-09) removed in the Probe content table, as well as the accompanying sentences below the table. - Probemix is now IVDR certified.

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