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	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 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	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

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) semiquantitative assay[2] for the detection of deletions or duplications in *SDHB*, *SDHC*, *SDHD*, *SDHAF1*, and *SDHAF2* 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.

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*, *SDHD*, *SDHAF1*, and *SDHAF2* 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.

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 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

It is estimated that 1.2-5.5% of patients with hereditary PGL/PCC have pathogenic CNVs in *SDHB*, *SDHC*, or *SDHD*, which include the known *SDHB* Dutch founder deletion in exon 3 and the *SDHB* Spanish founder deletion in exon 1 (also detectable by SDHB upstream probe) (Bayley et al. 2005, Bayley et al. 2009, Buffet et al. 2012), all of which can be detected with this probemix.

Analytical performance can be compromised by: SNVs or other polymorphisms 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.

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 (Exon 1)	142.3 **M**b	202	21768-L30666	Ø
1q23.3	*SDHC*	Upstream (Exon 1)	0.6 kb	142	07350-L16209	Ø
1q23.3	*SDHC*	Intron 1 (Exon 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 (Exon 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 (Exon 6)		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 (Exon 1)	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 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. Annotations of several probes with targets at the edge of or slightly outside the coding region, were altered. The exon numbering from the previous version of this Product Description is disclosed between brackets when a discrepancy is present.
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

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 D1-09 – 21 February 2025 (03S) - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Salt warning for probe 15741-L06981 has been removed. - Warning for probes targeting a sequence outside the known coding region added for probes 21768-L30666, 07350-L16209, 16964-L19962, 16977-L19975, 16972-L19970, and 07357-L1621. - Warning for probes with a ligation site >20nt from the nearest exon added for probes 16978-L19976, 14641-L16291, and 16962-L19960.