Product Description
SALSA® MLPA® Probemix P226-D1 SDH

To be used with the MLPA General Protocol.

Version D1
For complete product history see page 11.

Catalogue numbers:
- **P226-025R:** SALSA MLPA Probemix P226 SDH, 25 reactions.
- **P226-050R:** SALSA MLPA Probemix P226 SDH, 50 reactions.
- **P226-100R:** SALSA MLPA Probemix P226 SDH, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit and Coffalyser.Net data analysis software. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman/SCIEX capillary sequencers, respectively (see www.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at www.mrcholland.com.

Precautions and warnings
For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mrcholland.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 purpose
The SALSA MLPA Probemix P226 SDH is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative 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 in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).

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

Clinical background
Paragangliomas (PGLs) are neuroendocrine tumours that originate from neural crest-derived cells. They arise from sympathetic or parasympathetic paraganglia tissues and can be situated in the head and neck region, thorax, abdomen, and pelvis. Tumours that arise from the adrenal medulla are called pheochromocytomas

(PCCs). Symptoms of PGL/PCC result either from mass effects (for example carotid body enlargement, visible in the neck) or catecholamine hypersecretion. PGLs are rare (incidence of ca. 2 in 1.000.000 individuals), and are most frequently found in individuals between the age of 30 and 50 years. For PCCs, the true incidence is unknown, as many affected individuals remain undiagnosed.

The hereditary PGL/PCC syndromes are inherited in an autosomal dominant manner. Pathogenic variants in the succinate dehydrogenase (SDH) genes, including *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* (collectively referred to as *SDHx*) cause PGL/PCC and occur in up to 40% of cases. Probes for *SDHA* are not included in this P226 SDH probemix, but SALSA MLPA Probemix P429 SDHA-MAX-TMEM127 can be used to detect CNVs in the *SDHA* gene. *SDHx* genes are tumour suppressor genes and loss of heterozygosity is a second hit in tumours. *SDHD* and *SDHAF2* demonstrate parent-of-origin effects and generally cause disease only when the pathogenic variant is inherited from the father (Hao et al. 2009, Hensen et al. 2004), with a penetrance of 90% or higher by the age of 70. Mutations in *SDHA*, *SDHB* and *SDHC* are inherited in an autosomal dominant manner with no parent-of-origin effect and show a low penetrance (Benn et al. 2006). Mutations in the *SDHAF1* gene are a cause of the recessive disorder SDH defective infantile leukoencephalopathy (Ghezzi D et al. 2009), *SDHAF1* loss-of-function might also cause PGL due to the function of *SDHAF1* in the SDH complex. This association, however, has not yet been established.

It is estimated that 10-25% of hereditary PGL/PCC syndrome cases are caused by pathogenic variants in the *SDHB* gene, 2-8% in the *SDHC* gene, 8-9% in the *SDHD* gene. *SDHAF2* mutations are very rare (<0.1%). The majority of mutations in the *SDHx* genes are point mutations and small deletions. 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 founder mutations: *SDHB* Dutch founder deletion in exon 3 and the *SDHB* Spanish founder deletion in exon 1 (Bayley et al. 2005, 2009, Buffet et al. 2012), all of which can be detected with this probemix.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1548/.

Gene structure
The *SDHB* gene spans ~36 kilobases (kb) on chromosome 1p36.1 and contains 8 exons. The *SDHB* LRG_316 is available at www.lrg-sequence.org and is identical to GenBank NG_012340.1.

The *SDHC* gene spans ~61 kb on chromosome 1q23.3 and contains 6 exons. The *SDHC* LRG_317 is available at www.lrg-sequence.org and is identical to GenBank NG_012767.1.

The *SDHD* gene spans ~33 kb on chromosome 11q23.1 and contains 4 exons. The *SDHD* LRG_9 is available at www.lrg-sequence.org and is identical to GenBank NG_012337.3.

The *SDHAF1* gene spans ~1 kb on chromosome 19q13.12 and contains 1 exon. No LRG is available for *SDHAF1*.

The *SDHAF2* gene spans ~17 kb on chromosome 11q12.2 and contains 4 exons. The *SDHAF2* LRG_519 is available at www.lrg-sequence.org and is identical to GenBank NG_023393.1.

Transcript variants
For *SDHB,* one transcript variant has been described encoding the full length protein (NM_003000.3; 1015 nucleotides (nt); coding sequence (CDS) 14-856; https://www.ncbi.nlm.nih.gov/gene/6390). The ATG translation start site is located in exon 1 and the stop codon is located in exon 8.

For *SDHC,* multiple variants have been described. Transcript variant 1 encodes the longest isoform (1) (NM_003001.5.; 1308 nt; CDS 26-535; https://www.ncbi.nlm.nih.gov/gene/6391). The ATG translation start site is located in exon 1 and the stop codon is located in exon 6.

For *SDHD,* multiple variants have been described. Transcript variant 1 encodes the longest isoform (1) (NM_003002.4.; 1339 nt; CDS 36-515; https://www.ncbi.nlm.nih.gov/gene/6392). The ATG translation start site is located in exon 1 and the stop codon is located in exon 4.

For *SDHAF1,* one transcript variant has been described encoding the full length protein (NM_001042631.3; 1125 nt; CDS 88-435; https://www.ncbi.nlm.nih.gov/gene/644096). The ATG translation start and the stop codon are both located in exon 1.

For *SDHAF2,* one transcript variant has been described encoding the full length protein (NM_017841.4; 1186 nt; CDS 24-524; https://www.ncbi.nlm.nih.gov/gene/54949). The ATG translation start site is located in exon 1 and the stop codon is located in exon 4.

Exon numbering
The *SDHB* exon numbering used in this P226-D1 SDH product description is the exon numbering from the LRG_316 sequence. For *SDHC* the exon numbering from the LRG_317 and for *SDHD* the exon numbering from LRG_9 is used. For *SDHAF1*, no LRG is available and therefore the exon numbering is based on NM_001042631.3. For *SDHAF2*, the exon numbering from LRG_519 is used.

The exon numbering of the NM_ sequence that were used for determining a probe ligation sites do not always correspond to the exon numbering obtained from the LRG sequences. As changes to the databases can occur after release of this product description, the NM_ sequences and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P226-D1 SDH contains 45 MLPA probes with amplification products between 130 and 494 nt. This includes nine probes for the *SDHB* gene, ten probes for *SDHC*, seven probes for *SDHD*, two probes for *SDHAF1* and four probes for *SDHAF2*. In addition, 13 reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.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.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal 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.mrcholland.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 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
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. 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 different unrelated individuals who are from families without a history of PGL/PCC. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA15099, NA00803, NA17941 and NA20775, from the Coriell Institute have been tested with this P226-D1 probemix at MRC Holland and can be used as a positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

Sample ID	Altered target genes in P226-D1	Expected copy number alteration/mutation
NA20775	*SDHAF2*	Heterozygous duplication of exon 2-4 of *SDHAF2*
NA00803	*SDHC*	Heterozygous deletion of the complete *SDHC* gene
NA17941	*SDHC*	Heterozygous duplication of the complete *SDHC* gene
NA15099	*SDHD*	Heterozygous duplication of the complete *SDHD* gene

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 founder mutations *SDHB* Dutch founder deletion in exon 3 and the *SDHB* Spanish founder deletion in exon 1 (Bayley et al. 2005, 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.

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.mrcholland.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 the probes detecting autosomal sequences are allele copy numbers of 2 (normal), 1 (heterozygous deletion), or 3 (heterozygous duplication). The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65

Copy number status	Final ratio (FR)
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- 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. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *SDHB* gene. 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 (as described for *DMD* by 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 or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *SDHx* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P226 SDH.
- 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (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

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.

***SDHx* mutation databases**

Databases for all *SDHx* genes can be found at: https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in these databases. 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 SNVs and unusual results (e.g., a duplication of *SDHB* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P226-D1 SDH

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		Reference	*SDHB*	*SDHC*	*SDHD*	*SDHAF1*	*SDHAF2*
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 00797-L00463	5q					
136	**SDHAF1 probe** 14638-L16288					**Exon 1**	
142	**SDHC probe** 07350-L16209			**Exon 1**			
148	Reference probe 08578-L08579	17q					
155	**SDHC probe** 16961-L19959			**Exon 5**			
160	**SDHAF2 probe** 14639-L16289						**Exon 1**
166	**SDHAF1 probe** 21556-L30299					**Exon 1**	
172	**SDHD probe** 16962-L19960				**Exon 3**		
178	Reference probe 02958-L02390	7q					
190	**SDHC probe** 16964-L19962			**Exon 1**			
196	**SDHAF2 probe** 16965-L19963						**Exon 2**
202	**SDHB probe** 21768-L30666		**Exon 1**				
211	**SDHB probe** 11094-L30475		**Exon 3**				
220	**SDHD probe** 07361-L20367				**Exon 4**		
226	Reference probe 12269-L13212	22q					
232	**SDHB probe** 16967-L19965		**Exon 2**				
238	**SDHB probe** 07347-L06979		**Exon 6**				
244	**SDHD probe** 21557-L30298				**Exon 4**		
250	**SDHC probe** 07356-L30156			**Exon 6**			
256	Reference probe 09560-L30934	20p					
264	**SDHD probe** 21558-L30105				**Exon 3**		
270	**SDHC probe** 14641-L16291			**Exon 3**			
279	Reference probe 12437-L13438	14q					
286	**SDHC probe** 21559-L30106			**Exon 4**			
292 Δ	**SDHD probe** 16971-L19969				**Exon 1**		
303	Reference probe 05697-L05139	12q					
310 «	**SDHB probe** 15741-L06981		**Exon 8**				
319	**SDHC probe** 16972-L19970			**Exon 6**			
326	**SDHD probe** 07357-L16211				**Exon 1**		
336	Reference probe 05433-L04849	3p					
355	**SDHD probe** 16973-L19971				**Exon 2**		
364	**SDHC probe** 16974-L19972			**Exon 2**			
373	**SDHB probe** 14872-L16797		**Exon 4**				
384	**SDHC probe** 14642-L16292			**Exon 3**			
393	**SDHAF2 probe** 14643-L21022						**Exon 3**
400	Reference probe 07991-L07772	7q					
418	**SDHAF2 probe** 14646-L16296						**Exon 4**
427	**SDHB probe** 16976-L19974		**Exon 7**				
436	Reference probe 13340-L14766	18q					
445	**SDHC probe** 16977-L19975			**Exon 4**			
454	Reference probe 08274-L08153	8q					
463	**SDHB probe** 16978-L19976		**Exon 1**				
472	Reference probe 13413-L14870	6q					
483	**SDHB probe** 16980-L19978		**Exon 5**				
494	Reference probe 19137-L27130	21q					

[a] See section Exon numbering on page 3 for more information.

« 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.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Table 2. P226-D1 probes arranged according to chromosomal location

Table 2a. *SDHB* gene

Length (nt)	SALSA MLPA probe	*SDHB* exon[a]	Ligation site NM_003000.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
202	21768-L30666	Exon 1	197 nt before exon 1	ACCAAATGGGCA-TGCGCCGCTACT	0.1 kb
463	16978-L19976	Exon 1	73 nt before exon 1	GTCCTCAGTGGA-TGTAGGCTGGGC	9.3 kb
		start codon	*14-16 (Exon 1)*		
232	16967-L19965	Exon 2	122-123	CAGCCACAGCTC-CCCGTATCAAGA	11.7 kb
211	11094-L30475	Exon 3	245-246	ATGCTTTAATCA-AGATTAAGAATG	4.4 kb
373	14872-L16797	Exon 4	323-324	CTTGTGCAATGA-ACATCAATGGAG	0.9 kb
483	16980-L19978	Exon 5	465-466	TGCACAGTACAA-ATCCATTGAGCC	3.8 kb
238	07347-L06979	Exon 6	564-563, reverse	GAATGCACTCGT-AGAGCCCGTCCT	1.3 kb
427	16976-L19974	Exon 7	15 nt before exon 7	CTCAGCTAATCA-TCCCTGGTTTTC	3.8 kb
310 «	15741-L06981	Exon 8	821-822	TCAAGAAAATGA-TGGCAACCTATA	
		stop codon	*854-856 (Exon 8)*		

Table 2b. *SDHC* gene

Length (nt)	SALSA MLPA probe	*SDHC* exon[a]	Ligation site NM_003001.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
142	07350-L16209	Exon 1	388 nt before exon 1, reverse	TTGGCCGGTTGA-GACCCCGAAGAG	0.6 kb
		start codon	*26-28 (Exon 1)*		
190	16964-L19962	Exon 1	133 nt after exon 1	AGGCCAAGCGCT-CGGGGATCCTAG	9.1 kb
364	16974-L19972	Exon 2	49-50	TCTTGCAGACAC-GTTGGTCGTCAT	4.8 kb
384	14642-L16292	Exon 3	112-113	AGTGCTGTTCCT-TTGGGAACCACG	0.2 kb
270	14641-L16291	Exon 3	91 nt after exon 3	CTTCCCTCACTT-TTACTCAACCAA	12.1 kb
286	21559-L30106	Exon 4	266-265, reverse	CATATACATACC-TGCACTCAAAGC	0.1 kb
445	16977-L19975	Exon 4	106 nt after exon 4	CTTGATTTAGAG-GGAACAGTAAGT	16.1 kb
155	16961-L19959	Exon 5	422-423	GGAATGGGATCC-GACACTTGGTAA	5.5 kb
250	07356-L30156	Exon 6	441-442	GATGTGGGACCT-AGGAAAAGGCCT	1.0 kb
		stop codon	*533-535 (Exon 6)*		
319	16972-L19970	Exon 6	104 nt after exon 6	AATCTGACCTTT-ACCAGGAGGGAA	

Table 2c. *SDHD* gene

Length (nt)	SALSA MLPA probe	*SDHD* exon[a]	Ligation site NM_003002.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
326	07357-L16211	Exon 1	350 nt before exon 1	TTCGTGAGGGGA-ATGGGATGCAGC	0.3 kb
292 Δ	16971-L19969	Exon 1	18 nt before exon 1	GTGGGTGGGAAT-TGTCGCCTAAGT	1.1 kb
		start codon	*36-38 (Exon 1)*		
355	16973-L19971	Exon 2	5 nt after exon 2	CACCATTGTATG-TTCTCTCCATCG	0.9 kb
264	21558-L30105	Exon 3	215-216	GCTGGCTCCAAG-GCTGCATCTCTC	0.2 kb
172	16962-L19960	Exon 3	39 nt after exon 3	GTCTGCTCAGTT-TGTTTGCTGTGA	5.8 kb
244	21557-L30298	Exon 4	355-354, reverse	CAACTTGTCCAA-GGCCCCTAAAGA	0.5 kb
		stop codon	*513-515 (Exon 4)*		
220 #	07361-L20367	Exon 4	836-837	AAGAGAATCCAA-CTTTATTACGAT	

Table 2d. *SDHAF1* gene

Length (nt)	SALSA MLPA probe	*SDHAF1* exon[a]	Ligation site NM_001042631.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*88-90 (Exon 1)*		
166	21556-L30299	Exon 1	212-211, reverse	CGGACCGCGGCA-GGCCCGCATGCT	0.3 kb
		stop codon	*433-435 (Exon 1)*		
136	14638-L16288	Exon 1	553-554	AGCTTGACGAAT-TGGGGATGTCAG	

Table 2e. *SDHAF2* gene

Length (nt)	SALSA MLPA probe	*SDHAF2* exon[a]	Ligation site NM_017841.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*24-26 (Exon 1)*		
160	14639-L16289	Exon 1	48-49	CAGTGTTCTCGA-CTTCGTCGCTGG	7.5 kb
196	16965-L19963	Exon 2	149-150	AGCCCAACAGAT-TCCCAAAAGGAC	0.3 kb
393	14643-L21022	Exon 3	329-330	GAAAAGCAGCTG-AACCTCTATGAC	8.0 kb
418	14646-L16296	Exon 4	520-521	TGAAAAGCCACG-TTGAGCTGTGCT	
		stop codon	*522-524 (Exon 4)*		

[a] See section Exon numbering on page 3 for more information.

[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

« 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.

Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Single probe aberration(s) must be confirmed by another method.

Complete probe sequences are available at www.mrcholland.com.

Related SALSA MLPA probemixes

P429 SDHA-MAX-TMEM127	Contains probes for the *SDHA*, *MAX* and *TMEM127* genes.
P016 VHL	Contains probes for the *VHL* gene.
P198 FH	Contains probes for the *FH* gene.

References

- 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.
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P226 product history	
Version	*Modification*
D1	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.
C1	Fourteen target probes of *SDHB/SDHC/SDHD* have been replaced, one *SDHC* probe has been removed and two additional probes have been added for *SDHAF1* and *SDHAF2*. Furthermore, seven reference probes have been replaced and two have been added.
B2	The 88 and 96nt control fragments have been replaced (QDX2).
B1	Five target probes for *SDHB* and *SDHC* have been replaced, three *SDHC* probes have been added. Probes for *SDHAF1* and *SDHAF2* have been added.
A2	X and Y control fragments have been added at 100 and 105 nt.
A1	First release.

Implemented changes in the product description
Version D1-08 – 21 September 2023 (04P) - Coriell sample IDs in section Positive control DNA samples corrected. Version D1-07 – 13 September 2022 (04P) - Coriell samples NA00803 and NA17941 added to the list of Positive control DNA samples. - Warning about probe variability added to the 292 nt probe in Table 1 and 2. - Selected publications section updated. - Minor textual and layout changes. Version D1-06 – 03 February 2022 (04P) - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version D1-05 – 15 September 2021 (04P) - Product description rewritten and adapted to a new template. - Clinical background and Performance characteristics have been adjusted. - Ligation sites of the probes targeting the *SDHB*, *SDHC*, *SDHAF1* and *SDHAF2* genes have been updated according to new version of the NM_ reference sequence. - *References* and *Selected Publications* were curated and new literature was included. - UK has been added to the list of countries in Europe that accept the CE mark. Version D1-04 — 28 September 2020 (02P) - Product description rewritten and adapted to a new template. - Intended use changed to Intended purpose using new template text. - Ligation sites of the probes targeting the SDHD gene updated according to new version of the NM_ reference sequence: NM_003002.4. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene for SDHD probe 07361-L20367. - Updated section with selected publications. - Various minor textual or layout changes. Version D1-03 – 15 June 2020 (04) - Product is now registered for IVD use in Colombia and Israel.

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IVD	EUROPE* CE COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

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