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.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 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* genes 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 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 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. Both parasympathetic and sympathetic PGLs are rare. Estimates of the overall incidence of parasympathetic PGLs range from 1 in 30.000 to 1 in 100.000.

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*, cause PGL/PCC and occur in up to 40% of cases. Probes for *SDHA* are not included in this P226 SDH probemix, but are included in probemix P429 SDHA-MAX. SDH 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 SDH defective infantile leukoencephalopathy (Ghezzi D et al. 2009) and might cause PGL due to the function of *SDHAF1,* but this has not been reported.

Approximately 30% of hereditary PGL/PCC syndrome is caused by pathogenic variants in the *SDHD* gene, 22-38% in the *SDHB* gene, 4-8% in the *SDHC* gene, while for *SDHAF2* it is unknown. The majority of mutations in the SDH genes are point mutations and small deletions. It is estimated that around 5-17% of pathogenic mutations in the *SDHB*, *SDHC* and *SDHD* genes is attributed to large deletions/duplications, including the 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).

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

Gene structure: The *SDHB* gene (8 exons) spans ~36 kb of genomic DNA on chromosome 1p36.1. The *SDHB* LRG_316 is available at http://www.lrg-sequence.org and is identical to GenBank NG_012340.1.
The *SDHC* gene (6 exons) spans ~61 kb of genomic DNA and is located on chromosome 1q23.3. The *SDHC* LRG_317 is available at http://www.lrg-sequence.org and is identical to GenBank NG_012767.1.
The *SDHD* gene (4 exons) spans ~33 kb of genomic DNA and is located on chromosome 11q23.1. The *SDHD* LRG_9 is pending but available at http://www.lrg-sequence.org and is identical to GenBank NG_012337.3.
The *SDHAF1* gene (1 exon) spans ~1.1 kb on chromosome 19q13.12.
SDHAF2 gene (4 exons) spans ~17 kb on chromosome 11q12.2. The *SDHAF2* LRG_519 is available at http://www.lrg-sequence.org and is identical to GenBank NG_023393.1.

Transcript variants: For *SDHB* transcript variant NM_003000.2 (1161 nt, coding sequence 152-994) is used: https://www.ncbi.nlm.nih.gov/gene/6390.
For *SDHC* transcript variant NM_003001.3 (2858 nt, coding sequence 31-540) is used: https://www.ncbi.nlm.nih.gov/gene/6391.
For *SDHD* transcript variant NM_003002.4 (1339 nt, coding sequence 36-515) is used: https://www.ncbi.nlm.nih.gov/gene/6392.
For SDHAF1 transcript variant NM_001042631.2 (1147 nt, coding sequence 88-435) is used: https://www.ncbi.nlm.nih.gov/gene/644096.
For *SDHAF2* transcript variant NM_017841.2 (1227 nt, coding sequence 23-523) is used: https://www.ncbi.nlm.nih.gov/gene/54949. These sequences are all reference standards in the RefSeqGene project.

Exon numbering: The exon numbering used in this P226-D1 SDH product description for the *SDHB* gene is the exon numbering from the LRG_316 sequence. For the *SDHC* the exon numbering from the LRG_317 sequence was used. For *SDHD* the exon numbering is from the LRG_9 sequence. For *SDHAF1* the exon numbering is from the RefSeq transcript NM_001042631.2 (no LRG is available), and for *SDHAF2* the LRG_519 sequence was used.

Probemix content: The SALSA MLPA Probemix P226-D1 SDH contains 45 MLPA probes with amplification products between 130 and 494 nucleotides (nt). This includes 9 probes for the *SDHB* gene, 10 probes for *SDHC*, 7 probes for *SDHD*, 2 probes for *SDHAF1* and 4 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.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 nt and 96 nt 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 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 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.

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/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 NA15099 from the Coriell Institute has been tested with this P226-D1 probemix at MRC-Holland and can be used as a positive control sample to detect a whole gene duplication of *SDHD*. Sample ID number NA20775 from the Coriell Institute has been tested with this P226-D1 probemix at MRC-Holland and can be used as a positive control sample to detect a heterozygous duplication *SDHAF2* exon 2-4. The quality of cell lines can change; therefore samples should be validated before use.

Performance characteristics: 13.1% of all *SDHB*, 5% of all *SDHD*, and 16.7% of all *SDHC* pathogenic variants are large deletions that can be detected with this probemix (Buffet et al. 2012). The analytical sensitivity and specificity for the detection of deletions or duplications in the SDH genes is very high and can be considered >99% (based on a 2005-2020 literature study).

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 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.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 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 dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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
Ambiguous copy number	All other values

Please note that these above mentioned dosage quotients are only valid for germline testing. Dosage quotients are affected both by percentage of tumour cells and by possible subclonality.

- 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 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 SDH 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. 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: 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.

The SDH mutation database: Databases for all SDH genes can be found at: https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the SDH mutation 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 *SDHB* exons 1 and 3 but not exon 2) to MRC-Holland: info@mlpa.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					
154	**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	**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**				
382 *	**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 above section on exon numbering for more information.

* New in version D1 (from lot D1-0218 onwards).
¥ Changed in version D1 (from lot D1-0218 onwards). Minor alteration, no change in sequence detected.
« 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.

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_003000.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
202	21768-L30666	Exon 1	59 nt before exon 1	ACCAAATGGGCA-TGCGCCGCTACT	0.1 kb
463	16978-L19976	Exon 1	65-66	GTCCTCAGTGGA-TGTAGGCTGGGC	9.3 kb
		start codon	*152-154 (exon 1)*		
232	16967-L19965	Exon 2	260-261	CAGCCACAGCTC-CCCGTATCAAGA	11.7 kb
208	11094-L11781	Exon 3	383-384	ATGCTTTAATCA-AGATTAAGAATG	4.4 kb
373	14872-L16797	Exon 4	461-462	CTTGTGCAATGA-ACATCAATGGAG	0.9 kb
483	16980-L19978	Exon 5	603-604	TGCACAGTACAA-ATCCATTGAGCC	3.8 kb
238	07347-L06979	Exon 6	702-701 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	959-960	TCAAGAAAATGA-TGGCAACCTATA	
		stop codon	*992-994 (exon 8)*		

Table 2b. *SDHC* gene

Length (nt)	SALSA MLPA probe	SDHC Exon[a]	Ligation site NM_003001.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
142	07350-L16209	Exon 1	382 nt before exon 1, reverse	TTGGCCGGTTGA-GACCCCGAAGAG	0.6 kb
		start codon	*31-33 (exon 1)*		
190	16964-L19962	Exon 1	132 nt after exon 1	AGGCCAAGCGCT-CGGGGATCCTAG	9.1 kb
364	16974-L19972	Exon 2	54-55	TCTTGCAGACAC-GTTGGTCGTCAT	4.8 kb
382	14642-L16292	Exon 3	117-118	AGTGCTGTTCCT-TTGGGAACCACG	0.2 kb
270	14641-L16291	Exon 3	90 nt after exon 3	CTTCCCTCACTT-TTACTCAACCAA	12.1 kb
286	21559-L30106	Exon 4	271-270 reverse	CATATACATACC-TGCACTCAAAGC	0.1 kb
445	16977-L19975	Exon 4	105 nt after exon 4	CTTGATTTAGAG-GGAACAGTAAGT	16.1 kb
154	16961-L19959	Exon 5	427-428	GGAATGGGATCC-GACACTTGGTAA	5.5 kb
250	07356-L30156	Exon 6	446-447	GATGTGGGACCT-AGGAAAAGGCCT	1.0 kb
		stop codon	*538-540 (exon 6)*		
319	16972-L19970	Exon 6	1416-1417	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	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_001042631.2	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
136	14638-L16288	Exon 1	553-554	AGCTTGACGAAT-TGGGGATGTCAG	
		stop codon	*433-435 (exon 1)*		

Table 2e. *SDHAF2* gene

Length (nt)	SALSA MLPA probe	SDHAF2 Exon[a]	Ligation site NM_017841.2	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*23-25 (exon 1)*		
160	14639-L16289	Exon 1	47-48	CAGTGTTCTCGA-CTTCGTCGCTGG	7.5 kb
196	16965-L19963	Exon 2	148-149	AGCCCAACAGAT-TCCCAAAAGGAC	0.3 kb
393	14643-L21022	Exon 3	328-329	GAAAAGCAGCTG-AACCTCTATGAC	8.0 kb
418	14646-L16296	Exon 4	519-520	TGAAAAGCCACG-TTGAGCTGTGCT	
		stop codon	*521-523 (exon 4)*		

a) See above section on exon numbering for more information.

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.

« 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.
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.

Related SALSA MLPA probemixes

- P429 SDHA-MAX: Contains probes for most exons (10 of 15 exons) of *SDHA* and all exons of *MAX*.
- P016 VHL: Contains 9 probes for the *VHL* gene (two or more probes for each exon).
- P198 Fumarase deficiency (FH): Contains one probe for each exon of the *FH* gene.

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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 D1-04 — 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.

Version D1-02 – 29 June 2018 (04)
- Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version D1-01 – 04 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 1 and Table 2).

Version 19 – 12 January 2018 (55)
- Product description adapted to a new lot (new lot number added, new picture included).
- A new related product added on page 1.
- Minor textual changes.
- New references added on page 2.
- Ligation sites of the probes targeting the SDHD gene updated according to new version of the NM_reference sequence.

Version 18 – 29 February 2016 (55)
- Product description adapted to a new lot (new lot number added, new picture included).
- Related products added on page 1.

Version 17 – 05 February 2016 (55)
- Warning added in Table 1 and 2 for the 287 nt probe 16970-SP0420-L19968.
- Corrected text on data analysis.

Manufacturer's address adjusted.

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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). The product is for RUO in all other European countries.