Product Description
SALSA® MLPA® Probemix P429-C1 SDHA-MAX-TMEM127

To be used with the MLPA General Protocol.

Version C1
As compared to version B2, four probes for *TMEM127* and two probes for *SDHA* have been added, and one probe for *MAX* has been replaced. The flanking and reference probes have been revised completely. Several probes have been changed in length, but not in the sequence detected. For complete product history see page 10.

Catalogue numbers:
- **P429-025R:** SALSA MLPA Probemix P429 SDHA-MAX-TMEM127, 25 reactions.
- **P429-050R:** SALSA MLPA Probemix P429 SDHA-MAX-TMEM127, 50 reactions.
- **P429-100R:** SALSA MLPA Probemix P429 SDHA-MAX-TMEM127, 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.

General information
The SALSA MLPA Probemix P429 SDHA-MAX-TMEM127 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *SDHA*, *MAX* and *TMEM127* genes, which are associated with pheochromocytomas (PCCs) and paragangliomas (PGLs).

PCCs and PGLs are highly vascular, catecholamine-secreting tumours derived from chromaffin cells in the adrenal medulla (PCCs) and from extra-adrenal neural crest progenitors (PGLs). Although PCCs and PGLs are predominantly benign, 10-15% can develop metastases (Jimenez et al. 2013). Up to 40% of the PCC and PCL patients carry a germline mutation in one of the at least 12 genes, among them are the *SDHA*, *MAX* and *TMEM127* genes (Gimenez-Roqueplo et al. 2012; Neumannn et al. 2002). Eight out of the remaining susceptibility genes – *VHL, SDHB, SDHC, SDHD, NF1, RET* and *HRAS* – are included in other available SALSA MLPA Probemixes (see Related SALSA MLPA Probemixes on page 9).

Mutations in the succinate dehydrogenase complex subunit gene A (*SDHA*), at 5p13.33, can lead to PCC and PGL with variable penetrance (Burnichon et al. 2010). Moreover, bi-allelic *SDHA* mutations have been shown to cause an early onset neurodegenerative disorder known as Leigh syndrome (Hovarth et al. 2006).

Germline mutations in myc-associated factor X (*MAX*), at 14q23.2, have been shown to be responsible for ~1% of PCC/PGL patients (Comino-Mendez et al. 2011; Burnichon et al. 2012). The MAX transcription factor is a member of basic helix-loop-helix leucine zipper (bHLHZ) family of transcription factors. Through the ability of MAX to form homo- or heterodimers with other family members, like MYC and MAD, the *MAX* gene is suggested to act as a tumour suppressor gene and to play an important role in cell proliferation, differentiation and apoptosis (for review see Dang 2012; Cascon and Robledo 2012). Recently, both germline and somatic intragenic copy number alterations (CNAs) of *MAX* gene have been described (Korpershoek et al. 2016; Daly et al. 2018).

Truncating or missense germline mutations in transmembrane protein 127 (*TMEM127*), at 2q11.2, have been identified in PCC patients. Truncating or missense *TMEM127* mutations can lead to inactivation of the mTOR1 protein kinase (Qin et al. 2014) that regulates cell growth, cell proliferation, cell motility, cell survival, protein synthesis, autophagy and transcription (Lipton and Sahin 2014). Although pathways via which *TMEM127* acts are yet to be further elucidated, research has shown that the TMEM127 protein is a component of the mTORC1 lysosomal nutrient-sensing complex (Deng et al. 2018).

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

This SALSA MLPA probemix is not CE/FDA registered for use in diagnostic procedures. Purchase of this product includes a limited license for research purposes.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
For NM_ mRNA reference sequences: http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/
PhenoGram Plot: http://visualization.ritchielab.org/phenograms/plot

Exon numbering
The *SDHA* exon numbering used in this P429-C1 SDHA-MAX-TMEM127 product description is the exon numbering from the LRG_315 sequence, the *MAX* exon numbering is from LRG_530, and the *TMEM127* exon numbering is from LRG_528. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does 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_ sequence and exon numbering may not be up-to-date.

Probemix content
The SALSA MLPA Probemix P429-C1 SDHA-MAX-TMEM127 contains 45 MLPA probes with amplification products between 124 and 500 nucleotides (nt). This includes 12 probes for the *SDHA* gene, one probe targeting a region located upstream and two probes targeting a region located downstream of the *SDHA* gene; eight probes for the *MAX* gene, two probes targeting a region located upstream and two probes targeting a region located downstream of the *MAX* gene; four probes for the *TMEM127* gene, one probe targeting a region located upstream and one probe targeting a region downstream of the *TMEM127* gene. In addition, 12 reference probes are included that target relatively copy number stable regions in various cancer types including PCC and PGL. Partial probe sequences and the identity of the genes detected by the reference probes are available in table 3 and 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). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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, which includes DNA derived from paraffin-embedded tissues, 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. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

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 PCC or PGL. 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. The samples in the table below have been tested with this P429-C1 probemix at MRC Holland and can be used as positive control samples to detect duplications and deletions in the below mentioned chromosomal regions. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Chromosomal position of CNA*	Altered target and flanking genes in P429-C1	Expected CNA
NA10401	Coriell Institute	2q11.2	***TMEM127***, *STARD7, ITPRIPL1*	Heterozygous duplication
NA14131	Coriell Institute	5p15.33	***SDHA***, *CCDC127, PDCD6*	Heterozygous deletion
NA14523	Coriell Institute	5p15.33	***SDHA***, *CCDC127, PDCD6*	Heterozygous duplication
NA05966	Coriell institute	14q23.1-q24.3	***MAX***, *OTX2, RDH12, NPC2*	Heterozygous duplication

* Indicated chromosomal bands accommodate genes targeted by MLPA probes, however, the whole extent of CNA present in this cell line cannot be determined by this P429-C1 SDHA-MAX-TMEM127 probemix.

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 standard deviation of each individual probe over all the reference samples should be ≤0.10. When the probemix is used on germline samples, 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
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication/gain	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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios 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 or subclonal 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. 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, most genetic alterations in the *SDHA, MAX* and *TMEM127* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P429 SDHA-MAX-TMEM127.
- 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.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in solid tumours with more chaotic karyotypes.

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.

Mutation databases

***SDHA*:** https://databases.lovd.nl/shared/genes/SDHA
MAX: https://databases.lovd.nl/shared/genes/MAX
***TMEM127*:** https://databases.lovd.nl/shared/genes/TMEM127

We strongly encourage users to deposit positive results in the mutation databases mentioned above. In the case of somatic mutations found in tumour samples, we encourage to deposit positive results in the Cosmic database: http://cancer.sanger.ac.uk/cosmic. 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 in germline samples, false positive results due to SNVs and unusual results (e.g., a duplication of *TMEM127* exons 2 and 4 but not exon 3) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P429-C1 SDHA-MAX-TMEM127

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]			
		Reference	***SDHA***	***MAX***	***TMEM127***
64-105	Control fragments – see table in probemix content section for more information				
124 *	Reference probe 19616-L26275	4p13			
130 *	**TMEM127 probe** 23127-L32687				**Exon 1**
148 *	Reference probe 10663-L11245	6p12			
153 ¬	**PDCD6 probe** 15441-L17271		5p15.33		
160 * ¬	**ITPRIPL1 probe** 23122-L32682				2q11.2
166¬	**NFKBIA probe** 01063-L23482			14q13.2	
172 ¥	Reference probe 10922-L25079	9q34			
178 ± #	**SDHA probe** 18228-L23469		**Exon 7**		
186 ¬	**OTX2 probe** 10204-L23539			14q23.1	
193	**SDHA probe** 17915-L22220		**Exon 4**		
200 ¥	**MAX probe** 17916-L32744			**Exon 3**	
205 *	Reference probe 10880-L32745	15q11			
211 ¥ ±	**SDHA probe** 17917-L32746		**Exon 2**		
220 * #	**SDHA probe** 23123-L32683		**Exon 3**		
226 *	**TMEM127** probe 23130-L32690				**Exon 2**
232 ¬	**NPC2 probe** 11660-L21613			14q24.3	
240	**MAX probe** 17919-L23480			**Downstream**	
250 *	Reference probe 05386-L29196	12p11			
256 Ж	**SDHA probe** 18830-SP0705-L25307		**Exon 9**		
265	**MAX probe** 17920-L24332			**Exon 1**	
274	**MAX probe** 17921-L22226			**Exon 5**	
283 Ж Ø #	**SDHA probe** 17913-SP0570-L23479		**Intron 5**		
292 Ж #	**SDHA probe** 17922-SP0573-L22227		**Exon 12**		
301 *	Reference probe 22146-L31174	16p13			
309 #	**SDHA probe** 17923-L23474		**Exon 8**		
319 ± Ж Ø	**MAX probe** 17924-SP0574-L22229			**Intron 3**	
328 Ø #	**SDHA probe** 18390-L23377		**Intron 1**		
337 *	Reference probe 19093-L24980	4q35			
346 ¬	**CCDC127 probe** 02791-L17920		5p15.33		
355 * ¬	**STARD7 probe** 23124-L32684				2q11.2
364	**MAX probe** 17927-L23476			**Exon 2**	
372 ¬	**RDH12 probe** 08893-L23475			14q24.1	
379 *	Reference probe 05921-L05366	17q11			
391 Ж #	**SDHA probe** 18227-SP0575-L22230		**Exon 13**		
402 Ж #	**SDHA probe** 17929-SP0577-L22234		**Exon 11**		
409 *	Reference probe 14950-L16683	6q22			
418 Ø	**MAX probe** 17930-L22235			**Intron 4**	
436 *	**TMEM127 probe** 23132-L32692				**Exon 4**
445 ¬	**GFRA3 probe** 11152-L23481		5q31.2		
454 *	Reference probe 05916-L23023	21q11			
462 * #	**SDHA probe** 23125-L32747		**Exon 5**		
476 *	**MAX probe** 23126-L32686			**Exon 4**	
486 *	Reference probe 13539-L32748	19p13			
490 *	**TMEM127 probe** 23134-L32694				**Exon 3**
500 *	Reference probe 21229-L29604	10p11			

[a] See section Exon numbering on page 2 for more information.

* New in version C1.
¥ Changed in version C1. Minor alteration, no change in sequence detected.

± SNPs rs200374231, rs563937944 and rs201636829 could influence the signals of the probes at lengths 178, 211 and 319 nt, respectively. In case of apparent single probe deletions, it is recommended to sequence the region targeted by this probe.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.
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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 2. P429-C1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene/ Exon	Ligation site / Chromosomal position (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
***TMEM127* gene at 2q11.2** Ligation sites of the probes for *TMEM127* are indicated according to NM_017849.4.					
160 ¬	23122-L32682	*ITPRIPL1*	2q11.2 (telomeric)	TCTCTCAGCGGC-TCCAGGACATTC	62.1 kb
		start codon	*261-263 (exon 2)*		
130	23127-L32687	Exon 1	25-26	CCCCGGGTCTGT-CCGGGCGTTGCG	0.8 kb
226	23130-L32690	Exon 2	501-502	CGGACCTGCTGA-AAGGTGAGGGTG	10.2 kb
490	23134-L32694	Exon 3	545-546	CTCCTGCGGGTC-ATCGCCGCCTTC	0.9 kb
436	23132-L32692	Exon 4	716-717	TGGGCTTCTGAA-CTCATCTTGGCC	61.6 kb
		stop codon	*975-977 (exon 4)*		
355 ¬	23124-L32684	*STARD7*	2q11.2 (centromeric)	TGGAGACTCTGG-CACACTCGGATG	-
***SDHA* gene at 5p15.33** Ligation sites of the probes for *SDHA* are indicated according to NM_004168.4.					
346 ¬	02791-L17920	*CCDC127*	5p15.33 (telomeric)	ACGCCATGATCT-CAGAAAATCGGC	13.2 kb
		start codon	*37-39 (exon 1)*		
328 Ø #	18390-L23377	Intron 1	567 nt after exon 1	CGCCCGAGCTTA-GGCTTGCAGTTC	4.4 kb
211 ±	17917-L32746	Exon 2	70 nt before exon 2	TGCAAGGGGAAA-TTACTATCCCCC	1.2 kb
220 #	23123-L32683	Exon 3	137 nt after exon 3 reverse	AGACCCCTGAGA-GGGTGAGCTGAG	0.7 kb
193	17915-L22220	Exon 4	351-352	TGTTTCCAGGGA-GGAATCAATGCT	0.9 kb
462 #	23125-L32747	Exon 5	259 nt after exon 5	TACCAGGTTTTA-ACTTGGGATATG	1.5 kb
283 Ж Ø #	17913-SP0570-L23479	Intron 5	341 nt and 312 nt before exon 6	GGGCTGTGACTG-29 nt spanning oligo-GACTCCGAGTGG	3.0 kb
178 ± #	18228-L23469	Exon 7	44 nt before exon 7	ATAGGAGGTCCA-GATGTGGGCCGC	2.8 kb
309 #	17923-L23474	Exon 8	10 nt after exon 8	AGGTGCGTGTGA-TTTACCACCAGC	1.7 kb
256 Ж	18830-SP0705-L25307	Exon 9	1288-1289 and 20 nt after exon 9	CCACCAACTACA-28 nt spanning oligo-CCCACAGCTGGA	4.9 kb
402 Ж #	17929-SP0577-L22234	Exon 11	111 nt and 85 nt before exon 11	GGAGACTTACAG-26 nt spanning oligo-GCTACATGTTTG	10.9 kb
292 Ж #	17922-SP0573-L22227	Exon 12	39 nt and 16 nt after exon 12 reverse	CCTGCAGAAGGA-23 nt spanning oligo-CCCACTGTCTGC	0.4 kb
391 Ж #	18227-SP0575-L22230	Exon 13	42 nt and 67 nt after exon 13	TGCCTTTTCCTG-25 nt spanning oligo-CCTGCATTTTCT	59.8 kb
		stop codon	*2029-2031 (exon 15)*		
153 ¬	15441-L17271	*PDCD6*	5p15.33 (centromeric)	CTCTGACCAGTT-CCACGACATCCT	137.3 **M**b
445 ¬	11152-L23481	*GFRA3*	5**q**31.2	CACCTCTAGCAT-AAGCACCCCACT	-
***MAX* gene at 14q23.3** Ligation sites of the probes for *MAX* are indicated according to NM_002382.5.					
232 ¬	11660-L21613	*NPC2*	14q24.3 (telomeric)	CCGAGCTTGGAA-CTTCGTTATCCG	6.8 **M**b

Length (nt)	SALSA MLPA probe	Gene/ Exon	Ligation site / Chromosomal position (hg18)	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
372 ¬	08893-L23475	*RDH12*	14q24.1 (telomeric)	TCACTTGTGTAT-TTTGCTGCAGGA	2.6 **M**b
		start codon	*179-181 (exon 1)*		
265	17920-L24332	Exon 1	5 nt after exon 1	GAGCGACGTGAG-TCCTGGGGCTTC	0.7 kb
364	17927-L23476	Exon 2	235-236	CCGAGGTTTCAA-TCTGCGGTACGT	7.8 kb
200	17916-L32744	Exon 3	246-247	CTTCTAGGCTGA-CAAACGGGCTCA	6.1 kb
319 ± Ж Ø	17924-SP0574-L22229	Intron 3	5989 nt and 5962 nt before exon 4; NM_145114.3; 623-624 and 650-651	AGGAGCCATGGG-27 nt spanning oligo-TCCAGCCTCCTT	0.5 kb
476	23126-L32686	Exon 4	444-443 reverse	CATTCTGCCGCT-TGAGGTCGTCAA	9.8 kb
418 Ø	17930-L22235	Intron 4	513 nt after exon 4 reverse; NM_001320415.2; 503-502 reverse	ATTCCCCAGGAA-CAAAGAACTTGA	0.9 kb
274	17921-L22226	Exon 5	660-661	GGAGGCCAGCTA-AGCCACTCGGGG	70.2 kb
240	17919-L23480	Downstream	68.8 kb after exon 5 reverse; NM_197957.4; 355-354 reverse	AACTTCATCTTT-GTTCCCTGGGGA	8.2 **M**b
		stop codon	*659-661 (exon 5)*		
186 ¬	10204-L23539	*OTX2*	14q23.1 (centromeric)	TCCTGCATGCAG-AGGTCCTATCCC	21.4 **M**b
166 ¬	01063-L23482	*NFKBIA*	14q13.2 (centromeric)	CTGTAATGGCCG-GACTGCCCTTCA	-

[a] See section Exon numbering on page 2 for more information.

± SNPs rs200374231, rs563937944 and rs201636829 could influence the signals of the probes at lengths 178, 211 and 319 nt, respectively. In case of apparent single probe deletions, it is recommended to sequence the region targeted by this probe.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time. When this occurs in reference samples, it can look like an increased signal in the test samples.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
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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. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
124	19616-L26275	*ATP8A1*	4p13	CAGATTCTTCTT-CGAGGAGCTCAG	04-042,278
337	19093-L24980	*F11*	4q35	ATAGCTGGTGAA-TTGAGTCCCTGA	04-187,434
148	10663-L11245	*PKHD1*	6p12	CTCTAACCAGAT-AACATGTCCACG	06-052,060
409	14950-L16683	*LAMA2*	6q22	GATATGAAGGCC-AGTACTGTGAAA	06-129,712
172	10922-L25079	*SETX*	9q34	CTTCTGCAGACT-GCTGGAAGAGAA	09-134,143
500	21229-L29604	*CCDC7*	10p11	ATCGCCTTAAAC-AGAGGTCTAAAT	10-032,800
250	05386-L29196	*PKP2*	12p11	CACTTTGACACA-TACCACAGACAG	12-032,922
205	10880-L32745	*UBE3A*	15q11	CTACCACCAGTT-AACTGAGGGCTG	15-023,172
301	22146-L31174	*ABCA3*	16p13	CAGCAATTCTGG-GCCATGTTCCTG	16-002,278
379	05921-L05366	*WSB1*	17q11	TGTCAATCCGAA-GAGTGATGCCCA	17-022,663
486	13539-L32748	*CACNA1A*	19p13	GCCATTACATCC-TGAACCTGCGCT	19-013,255
454	05916-L23023	*HSPA13*	21q11	ATTCAGCAAGTA-TTGAAAGAAGGC	21-014,668

Related SALSA MLPA probemixes

P226 SDH	Contains probes for each exon of the *SDHD, SDHB, SDHC* genes.
P016 VHL	Contains nine probes for the *VHL* gene (two or more probes for each exon).
P169 Hirschsprung-1	Contains probes for all twenty exons of the *RET* gene.
P081 and P082 NF1 mix 1 and 2	Together contain probes for all exons of the *NF1* gene.
P298 BRAF-HRAS-KRAS-NRAS	Contains probes for each exon of the *HRAS* gene.

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Selected publications using SALSA MLPA Probemix P429 SDHA-MAX-TMEM127

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- Villabona C et al. (2021). The recurrent p.(Pro540Ser) *MEN1* genetic variant should be considered nonpathogenic: A case report. *Am J Med Genet A.* 1-5.

P429 product history	
Version	*Modification*
C1	Four probes targeting *TMEM127* and two flanking probes have been added, two new probes targeting *SDHA* have been added, one *MAX* probe has been replaced, one flanking probe for *SDHA* and one flanking probe for *MAX* have been removed, and the reference probe set has been revised completely.
B2	One reference probe has a small change in length but no change in sequence detected.
B1	One probe for *SDHA* exon 9 and two flanking probes for *SDHA* added, the exon 3 probe for *SDHA* removed and two reference probes replaced.
A1	First release.

Implemented changes in the product description

Version C1-02 – 10 October 2025 (04P)
- The warnings about SNPs possibly affecting probe signals have been adjusted in Table 1 and Table 2, since the information in the previous version of this product description was incorrect.
- Adjusted remarks regarding availability of complete probe sequences.
- Various minor textual changes.

Version C1-01 – 13 October 2021 (04P)
- Product description is completely rewritten and adapted to a new template, and to a new product version (version number changed, changes in Table 1, Table 2 and Table 3).
- Product name has been changed from P429 SDHA-MAX to P429 SDHA-MAX-TMEM127.
- Warnings added to Table 1 and Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.
- New positive sample added to page 3 for the *TMEM127* gene.
- Related SALSA MLPA probemixes is updated (P298 was added).
- The list of selected publications using P429 is updated.

Version B2-01 – 29 October 2020 (02P)
- Product description rewritten and adapted to a new template.
- Positive control DNA samples section added on pages 2-3.
- P429 specific notes added on page 4.
- Ligation site for the probes targeting the *SDHA* gene updated according to the new version of the NM_004168.4 reference sequence.
- Exon numbering of the *SDHA* and *MAX* gene has been changed.
- Ligation sites of the probes targeting the *MAX* gene updated according to new version of the NM_002382.5 reference sequence.
- Ligation sites of the probes targeting the *SDHA* gene updated according to new version of the NM_004168.4 reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- One new publication using SALSA MLPA Probemix P429 SDHA-MAX added on page 8.
- Various minor textual or layout changes.

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