Product Description SALSA® MLPA® Probemix P169-C2 Hirschsprung-1

To be used with the MLPA General Protocol.

Version C2. For complete product history see page 8.

Catalogue numbers:
- **P169-025R:** SALSA MLPA Probemix P169 Hirschsprung-1, 25 reactions.
- **P169-050R:** SALSA MLPA Probemix P169 Hirschsprung-1, 50 reactions.
- **P169-100R:** SALSA MLPA Probemix P169 Hirschsprung-1, 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.

General information: The SALSA MLPA Probemix P169 Hirschsprung-1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *RET*, *ZEB2*, *EDN3* and *GDNF* genes, which are associated with Hirschsprung disease (HSCR). HSCR or aganglionic megacolon, is a congenital disorder characterised by the absence of enteric ganglia along a variable length of the intestine. The disease can be classified according to the length of the aganglionosis into short segment (accounts for 80% of the HSCR cases), long segment and total colonic aganglionosis forms. Dominant mutations in the *RET* gene, as well as recessive mutations in several other genes including the *ZEB2*, *EDN3*, and *GDNF* genes, are associated with HSCR.

The *RET* gene encodes for one of the cell-surface receptor tyrosine kinases, which plays a role in cell growth and differentiation. *RET* is composed of 20 exons, spans ~53 kb of genomic DNA, and is located on 10q11.21, ~43 Mb from the p-telomere. The *ZEB2* gene encodes for a member of the Zfh1 family and functions as a DNA-binding transcriptional repressor that interacts with activated SMADs (SMADs are the main signal transducers for receptors of the TGF-β superfamily). The *EDN3* gene encodes for a member of the endothelin family. *EDN3* consists of 5 exons, spans ~26 kb of genomic DNA, and is located on 20q13.32, ~59 Mb from the p-telomere. The *GDNF* gene encodes for a neurotrophic factor. *GDNF* consists of 3 exons, spans ~27 kb of genomic DNA and is located on 5p13.2, ~38 Mb from the p-telomere.

More information on HSCR is available at https://www.ncbi.nlm.nih.gov/books/NBK1439/.

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/

Exon numbering: The *RET* exon numbering used in this P169-C2 Hirschsprung-1 product description is the exon numbering from the LRG_518 sequence, which is identical to the exon numbering of NG_007489.1. No LRG is available for *ZEB2*, therefore the exon numbering of the NG_016431.1 sequence is used, which is a reference standard in the RefSeqGene project. The *EDN3* LRG is still pending, therefore the exon numbering of the NG_008050.1 sequence is used, which is a reference standard in the RefSeqGene project. No LRG is

available for *GDNF,* therefore the exon numbering of the NG_011675.2 sequence is used, which is a reference standard in the RefSeqGene project. The exon numbering and NG_ sequences used have been retrieved in November 2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P169-C2 Hirschsprung-1 contains 51 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 12 probes for the *ZEB2* gene, five probes for *GDNF*, 20 probes for *RET*, and five probes for *EDN3*. In addition, nine 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, 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 HSCR. 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. The quality of cell lines can change; therefore samples should be validated before use.

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 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

- 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 *RET* and *ZEB2* genes. 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 *ZEB2*, *GDNF*, *RET*, and *EDN3* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P169 Hirschsprung-1.
- 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.

Mutation databases: For all four genes in this P169-C2 Hirschsprung-1 probemix mutation databases can be found on https://www.lovd.nl/. We strongly encourage users to deposit positive results in the LOVD 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 SNPs and unusual results (e.g., a duplication of *RET* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P169-C2 Hirschsprung-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*ZEB2*	*GDNF*	*RET*	*EDN3*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 16316-L18705	3q21				
136 «	**ZEB2** probe 05478-L04901		**Exon 1**			
141	**RET** probe 18025-L06390				**Exon 4**	
148	Reference probe 16544-L19035	11q13				
154 «	**RET** probe 05505-L05592				**Exon 11**	
160	**ZEB2** probe 05485-L04908		**Exon 6**			
166 «	**GDNF** probe 05515-L04938			**Exon 1**		
172 «	**ZEB2** probe 18078-L23060		**Exon 2**			
178 «	**RET** probe 05506-L04929				**Exon 12**	
183 « ±	**RET** probe 18079-L23061				**Exon 19a**	
190	**ZEB2** probe 18080-L22486		**Exon 3**			
196	**RET** probe 05497-L04920				**Exon 3**	
202	**RET** probe 18026-L22380				**Exon 6**	
208	Reference probe 10730-L11312	6p12				
214	**ZEB2** probe 05486-L22381		**Exon 7**			
220 «	**RET** probe 05507-L22379				**Exon 13**	
226 « +	**GDNF** probe 05516-L22382			**Intron 1**		
232 «	**RET** probe 05508-L20172				**Exon 14**	
238 «	**RET** probe 18027-L23566				**Exon 1**	
244	**ZEB2** probe 05481-L22731		**Exon 3**			
250	**ZEB2** probe 05487-L22732		**Exon 8**			
256	**RET** probe 05499-L23570				**Exon 5**	
262	Reference probe 17579-L23911	7q11				
268 «	**RET** probe 05509-L22733				**Exon 15**	
275	**ZEB2** probe 05483-L23064		**Exon 4**			
281	**EDN3** probe 18028-L23147					**Exon 1**
286	**EDN3** probe 05492-L22734					**Exon 3**
292	**RET** probe 18081-L23071				**Exon 2**	
300	Reference probe 16027-L18204	12p13				
307 Ж	**EDN3** probe 18082-SP0606-L22488					**Exon 2**
317 #	**ZEB2** probe 05488-L22735		**Exon 9**			
324	**GDNF** probe 12957-L22736			**Exon 2**		
331 ± Ж	**RET** probe 18331-SP0609-L23070				**Exon 7**	
338 «	**RET** probe 07288-L22737				**Exon 17**	
347	**ZEB2** probe 05484-L22740		**Exon 5**			
355	**EDN3** probe 05493-L23065					**Exon 4**
364 Ж	**RET** probe 18084-SP0607-L22490				**Exon 9**	
373 ¥	**RET** probe 18546-L28617				**Exon 8**	
381	Reference probe 12940-L23154	13q14				
389	**RET** probe 18029-L04927				**Exon 10**	
397 #	**ZEB2** probe 05489-L23067		**Exon 10**			
406	**GDNF** probe 05518-L23068			**Exon 3**		
416 «	**ZEB2** probe 06123-L22745		**Exon 1**			
422	Reference probe 10394-L22748	9q34				
433 «	**RET** probe 18030-L23567				**Exon 18**	
444	**EDN3** probe 05494-L23569					**Exon 5**
452 «	**RET** probe 05514-L23069				**Exon 20**	
460 « Ж +	**GDNF** probe 18085-SP0608-L22491			**Intron 1**		
469 «	**RET** probe 18330-L23098				**Exon 16**	
479	Reference probe 13539-L23528	19p13				
490	Reference probe 12461-L21828	22q12				

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

¥ Changed in version C2. Minor alteration, no change in sequence detected.

± SNP rs200289472 could influence the 183 nt probe signal (18079-L23061). SNP rs199572076 could influence the 331 nt probe signal (18331-SP0609-L23070). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
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.
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.
+ These probes target alternative exons not present in the RefSeqGene standard transcript NM_000514.4; their target sites are located in intron 1 of NG_008050.1. The 226 nt probe targets exon 1 of transcript variant NM_199231.2 and the 460 nt probe targets exon 1 of transcript variants NM_001190468.1 and transcript NM_001190469.1. The significance of deletions/duplications of these alternative exons only is not clear, as they are is non-coding and other transcript variants using different transcription start sites are known.

Table 2. P169-C2 probes arranged according to chromosomal location

Table 2a. *ZEB2*

Length (nt)	SALSA MLPA probe	*ZEB2* exon[a]	Ligation site NM_014795.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
136 «	05478-L04901	Exon 1	196 nt before exon 1	CAGAGAGAAACT-TGGCGATCACGT	0.3 kb
416 «	06123-L22745	Exon 1	116-117	CTCCCCCACACT-TCGCGGCTTCTT	2.6 kb
172 «	18078-L23060	Exon 2	202-203	GCGTGCTGCCGA-AGCAGGGCGCCG	87.4 kb
		start codon	*251-253 (exon 2)*		
244	05481-L22731	Exon 3	355-356	GTGGACACAGGT-TCTGAAACAGAT	0.1 kb
190	18080-L22486	Exon 3	450-451	TAGTGTGCCCAA-CCATGAGTCCTC	5.1 kb
275	05483-L23064	Exon 4	619-620	ATGGGGCCAGAA-GCCACGATCCAG	19.9 kb
347	05484-L22740	Exon 5	782-783	TTATTTACCCAG-AAGCCCCTGAGG	0.9 kb
160	05485-L04908	Exon 6	1019-1020	GCACCCAGCTCG-AGCGGCATATGG	2.7 kb
214	05486-L22381	Exon 7	1084-1085	CAAGGAGCAGGT-AATCGCAAGTTC	2.5 kb
250	05487-L22732	Exon 8	2663-2664	ACACTCCAAACA-GCTTCTCTTCTG	2.3 kb
317 #	05488-L22735	Exon 9	3270-3271	ATGTGACAAGAC-ATTCCAGAAAAG	7.8 kb
		stop codon	*3893-3895 (exon 10)*		
397 #	05489-L23067	Exon 10	4643-4644	TGTTAAGAGGGT-AACATGGGTTAC	

Table 2b. *GDNF*

Length (nt)	SALSA MLPA probe	*GDNF* exon[a]	Ligation site NM_000514.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
166 «	05515-L04938	Exon 1	463-464	ATCAGCCCGGAT-GGGTCTCCTGGC	3.8 kb
460 « Ж +	18085-SP0608-L22491	Intron 1	NM_001190468.1; 49-50 and 79-80	GACGGGGGCGCG-30 nt spanning oligo-GGATTAGGGCCA	0.3 kb
226 « +	05516-L22382	Intron 1	NM_199231.2; 28-29	CTCCAAGTCCCT-GCTAACTTCTTG	0.7 kb
		start codon	*562-564 (exon 2)*		
324	12957-L22736	Exon 2	571-570 *reverse*	CACGACATCCCA-TAACTTCATCTT	19.0 kb
406	05518-L23068	Exon 3	1082-1083	GAGTGACAAAGT-AGGGCAGGCATG	
		stop codon	*1195-1197 (exon 3)*		

Table 2c. *RET*

Length (nt)	SALSA MLPA probe	*RET* exon[a]	Ligation site NM_020975.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
238 «	18027-L23566	Exon 1	166-167	CAGTCCCTCCAG-CCGTGGCCCCAG	23.4 kb
		start codon	*191-193 (exon 1)*		
292	18081-L23071	Exon 2	482-483	TCCTCTACCTTA-ACCGGAGCCTGG	1.9 kb
196	05497-L04920	Exon 3	773-774	TGCCTGTGCAGT-TCTTGTGCCCCA	2.4 kb
141	18025-L06390	Exon 4	836-837	TGCCCTTCCGCT-GCGCCCCGGACA	1.5 kb
256	05499-L23570	Exon 5	1201-1202	TGGCCCAACGAG-ACCTCGGTCCAG	2.6 kb
202	18026-L22380	Exon 6	1299-1300	GAACCGCACCAT-GCAGCTGGCGGT	2.4 kb
331 ± Ж	18331-SP0609-L23070	Exon 7	1689-1690 and 8 nt after exon 7	CCAGGCCCAGCT-32 nt spanning oligo-TGCTCCAGGGAG	0.7 kb
373	18546-L28617	Exon 8	1758-1759	TGCAGTCAGCAA-GAGACGGCTGGA	0.7 kb
364 Ж	18084-SP0607-L22490	Exon 9	50 nt and 20 nt before exon 9	GGTGGTGGGGGC-30 nt spanning oligo-CTGCTGTGTGTC	0.8 kb
389	18029-L04927	Exon 10	2024-2025	CCTGCAACTGCT-TCCCTGAGGAGG	0.9 kb
154 «	05505-L05592	Exon 11	2153-2154	TGCTGTCTGCCT-TCTGCATCCACT	2.0 kb
178 «	05506-L04929	Exon 12	2351-2352	GGGAATTCCCTC-GGAAGAACTTGG	1.8 kb
220 «	05507-L22379	Exon 13	2514-2515	CCTGCTGTCAGA-GTTCAACGTCCT	1.3 kb
232 «	05508-L20172	Exon 14	2752-2753	CTCATCTCATTT-GCCTGGCAGATC	0.4 kb
268 «	05509-L22733	Exon 15	2823-2824	CTTGGCAGCCAG-AAACATCCTGGT	1.9 kb
469 «	18330-L23098	Exon 16	2983-2984	TACACCACGCAA-AGTGATGTGTAA	1.7 kb
338 «	07288-L22737	Exon 17	2999-3000	GCAGATGGTCTT-TTGGTGTCCTGC	1.2 kb
433 «	18030-L23567	Exon 18	3155-3156	TGCAATGCTGGA-AGCAGGAGCCGG	1.8 kb
183 ± «	18079-L23061	Exon 19a	3354-3355	CCTCCCTTCCAC-ATGGATTGAAAA	2.4 kb
		stop codon	*3533-3535 (exon 20)*		
452 «	05514-L23069	Exon 20	4383-4384	CCCAGAATTGCT-GACAGCAGAGGC	

Table 2d. *EDN3*

Length (nt)	SALSA MLPA probe	*EDN3* exon[a]	Ligation site NM_207034.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
281	18028-L23147	Exon 1	24-25	GCAGCGCGCTCT-GAAAGTTTATGA	0.8 kb
		start codon	*203-205 (exon 1)*		
307 Ж	18082-SP0606-L22488	Exon 2	310-309 and 286-285, reverse	GCTGCAGTGGGG-24 nt spanning oligo-GCATCCCCAGAC	19.7 kb
286	05492-L22734	Exon 3	636-637	TGCGGGGCCACT-TCCAGGGAATCT	1.3 kb
355	05493-L23065	Exon 4	778-779	ACAGACAAAGAA-GAGGAAGGGAAG	3.5 kb
		stop codon	*917-919 (exon 5)*		
444	05494-L23569	Exon 5	2360-2361	GTTTGGCACCGT-GGCAAGATGGTA	

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.

± SNP rs200289472 could influence the 183 nt probe signal (18079-L23061). SNP rs199572076 could influence the 331 nt probe signal (18331-SP0609-L23070). In case of apparent deletions, it is recommended to sequence the region targeted by this probe.
« 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.
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.
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.
+ These probes target alternative exons not present in the RefSeqGene standard transcript NM_000514.4; their target sites are located in intron 1 of NG_008050.1. The 226 nt probe targets exon 1 of transcript variant NM_199231.2 and the 460 nt probe targets exon 1 of transcript variants NM_001190468.1 and

transcript NM_001190469.1. The significance of deletions/duplications of these alternative exons only is not clear, as they are is non-coding and other transcript variants using different transcription start sites are known.

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

Related SALSA MLPA probemixes

- P318 Hirschsprung-2: Contains probes for the *PHOX2B*, *GFRA3*, *GFRA2*, *GFRA1*, *EDNRB*, *NRTN*, *PSPN* and *SOX10* genes.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

Selected publications using SALSA MLPA Probemix P169 Hirschsprung-1

- Bim LV et al. (2019). Retroposed copies of RET gene: a somatically acquired event in medullary thyroid carcinoma. *BMC Med Genomics.* 12:104.
- Meral C et al. (2012). A case of Mowat-Wilson syndrome caused by a truncating mutation within exon 8 of the ZEB2 gene. *Turk J Pediatr.* 54:523-527.
- Núñez-Torres, R. et al. (2009) A novel study of Copy Number Variations in Hirschsprung disease using the Multiple Ligation-dependent Probe Amplification (MLPA) technique. *BMC Med Genet*. 10: 119.
- Sánchez-Mejías, A. et al. (2010) Novel MLPA procedure using self-designed probes allows comprehensive analysis for CNVs of the genes involved in Hirschsprung disease. *BMC Med Genet*. 11: 71.

P169 Product history	
Version	*Modification*
C2	The length of one probe has been adjusted.
C1	Two new probes for *ZEB2*, two new probes for *GDNF*, eight new probes for *RET*, two new probes for *EDN3* have been added, nine reference probes are added and new control fragments (QDX2) have been included.
B1	Two *RET* probes (exon 17, 19) have been replaced. The *RET* exon 18 probe has been modified, no change in sequence detected. Extra control fragments at 88, 96, 100, 105 nt have been added.
A1	First release.

Implemented changes in the product description

Version C2-01 — 15 November 2019(02P)
- Product description rewritten and adapted to a new template.
- Exon numbering of all genes is now according to the most recent information and current standards.
- Ligation sites of the probes targeting *ZEB2*, *GDNF* and *EDN3* has been updated according to new version of the NM_ reference sequences.
- Warning added to Table 1 and 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

Version 12 – 12 November 2015 (55)
- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).
- Various exon numberings changed.
- Refseq sequence and ligation sites EDN3 adjusted.

Version 11 – 01 July 2015 (54)
- Figure based on the use of old MLPA buffer (replaced in December 2012) removed.

Version 10 (49)

- Product description adapted to a new product version (version number changed, lot number added, changes in Table 1 and Table 2, new picture included).

More information: www.mlpa.com; www.mlpa.eu	
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