Product Description SALSA® MLPA® probemix P319-B1 Thyroid

To be used with the MLPA General Protocol.

Version B1. As compared to version A2, one probe for *PAX8* and one for *TSHR* have been removed, three reference probes have been replaced and several probe lengths have been adjusted. For complete product history see page 6.

Catalogue numbers:
- **P319-025R:** SALSA® MLPA® probemix P319 Thyroid, 25 reactions.
- **P319-050R:** SALSA® MLPA® probemix P319 Thyroid, 50 reactions.
- **P319-100R:** SALSA® MLPA® probemix P319 Thyroid, 100 reactions.

To be used in combination with a SALSA® MLPA® reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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 P319 Thyroid is a **research use only (RUO)** assay for the detection of deletions or duplications in the *TPO*, *PAX8*, *FOXE1*, *NKX2-1* and *TSHR* genes. Thyroid dysgenesis (TD) accounts for most cases of congenital hypothyroidism. TD is generally a sporadic disease, but in about 5% of the cases a genetic origin has been demonstrated. In these cases, mutations in genes playing a role during thyroid morphogenesis (*PAX8*, *FOXE1*, *NKX2-1*, *TSHR*) have been reported.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK279032/

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/

Probemix content: The SALSA MLPA Probemix P319-B1 Thyroid contains 49 MLPA probes with amplification products between 127 and 481 nt.
The *TPO* gene (17 exons) spans ~129 kb of genomic DNA and is located on 2p25.3, ~1.4 Mb from the p-telomere. This probemix contains one probe for each exon of the *TPO* gene (two probes for exon 8) with the exception of exon 2 and 3.
The *PAX8* gene (10 exons) spans ~63 kb of genomic DNA and is located on 2q14.1, ~113 Mb from the p-telomere. This probemix contains one probe for each exon of the *PAX8* gene (two probes for exon 1) with the exception of exon 3 and 6.
The *FOXE1* gene (1 exon) spans ~3.5 kb of genomic DNA and is located on 9q2233, ~98 Mb from the p-telomere. This probemix contains two probes for *FOXE1*.
The *NKX2-1* gene (3 exons) spans ~4 kb of genomic DNA and is located on 14q13.3, ~37 Mb from the p-telomere. This probemix contains probes for each exon of the *NKX2-1* gene.
The *TSHR* gene (10 exons) spans ~191 kb of genomic DNA and is located on 14q31.1, ~81 Mb from the p-telomere. This probemix contains one probe for each exon of the *TSHR* gene.

In addition, nine reference probes are included in this probemix, detecting nine different autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes is 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, 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 or 96 fragment indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

No DNA controls results in only five major peaks shorter than 121 nucleotides (nt): four Q-fragments at 64, 70, 76 and 82 nt, and one 19 nt peak corresponding to the unused portion of the fluorescent PCR primer. Non-specific peaks longer than 121 nt AND with a height >25% of the median of the four Q-fragments should not be observed. Note: peaks below this 25% threshold are not expected to affect MLPA reactions when sufficient amount of sample DNA (50-200 ng) is used.

MLPA technique: The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mlpa.com).

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: 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 thyroid abnormalities. 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 Biobank (https://catalog.coriell.org) and 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 must 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 all probes in the reference samples should be <0.10 and the dosage quotient (DQ) of the reference probes 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 or pseudo-autosomal chromosomes:

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. 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 (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 in some cases not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Limitations of the procedure:
- In most populations, the major cause of genetic defects in the *TPO*, *PAX8*, *FOXE1*, *NKX2*-1 and *TSHR* genes are small (point) mutations, none of which will not be detected by using SALSA® MLPA® Probemix P319 Thyroid.
- 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 one or more than one consecutive probe (Table 2) 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.

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 *TPO* exons 6 and 8 but not exon 7) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P319-B1 Thyroid

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)					
		Reference	*TPO*	*PAX8*	*FOXE1*	*NKX2-1*	*TSHR*
64-105	Control fragments – see table in probemix content section for more information						
127 *	Reference probe 18709-L26552	5q31					
130	**TPO probe** 10913-L11582		**Exon 1**				
136	**NKX2-1 probe** 10917-L14480					**Exon 1**	
142	**TPO probe** 10920-L14478		**Exon 15**				
148 *	Reference probe 15691-L17658	1p34					
154	**TSHR probe** 10928-L11597						**Exon 1**
160	**PAX8 probe** 10931-L11600			**Exon 1**			
166	**TSHR probe** 10934-L11604						**Exon 10**
172	**PAX8 probe** 10971-L11642			**Exon 2**			
178	**TPO probe** 10972-L11643		**Exon 11**				
184	**TSHR probe** 10973-L11644						**Exon 5**
190	**PAX8 probe** 10974-L15609			**Exon 9**			
196	**TSHR probe** 10975-L11646						**Exon 7**
202	**PAX8 probe** 10977-L11648			**Exon 1**			
208	**NKX2-1** probe 10978-L11649					**Exon 3**	
214	Reference probe 10338-L09519	11q13					
220	**PAX8 probe** 10981-L14482			**Exon 10**			
226 ¥	Reference probe 10672-L31279	6p12					
231 ¥	**TPO probe** 22021-L31280		**Exon 6**				
238	**FOXE1 probe** 11038-L11706				**Exon 1**		
244	**TSHR probe** 11039-L11707						**Exon 2**
250	**TPO probe** 11040-L15610		**Exon 16**				
256	**PAX8 probe** 12810-L14483			**Exon 8**			
260	Reference probe 13529-L14989	3q21					
267	**FOXE1 probe** 11042-L16031				**Exon 1**		
274	**TSHR probe** 11043-L11711						**Exon 3**
283	**TPO probe** 11044-L11713		**Exon 8**				
292	**TSHR probe** 11045-L11714						**Exon 9**
301	**TPO probe** 11046-L11715		**Exon 12**				
310	**TSHR probe** 12809-L14484						**Exon 4**
319	**TPO probe** 05845-L14485		**Exon 4**				
325 *	Reference probe 05971-L21639	7p11					
337	**TPO probe** 11049-L11718		**Exon 7**				
346	**PAX8 probe** 11050-L14486			**Exon 7**			
355	**NKX2-1 probe** 11051-L14487					**Exon 2**	
364	Reference probe 09775-L10190	15q21					
373	**TPO probe** 11052-L11721		**Exon 9**				
382	**TPO probe** 13469-L11723		**Exon 13**				
391	Reference probe 12762-L13878	4q12					
400	**PAX8 probe** 11055-L11724			**Exon 5**			
409	**TPO probe** 11056-L15926		**Exon 5**				
418	**TSHR probe** 11057-L11726						**Exon 8**
426	**TPO probe** 11058-L15927		**Exon 10**				
436	**TPO probe** 13473-L11733		**Exon 17**				
444	**TPO probe** 13472-L11732		**Exon 8**				
453	**PAX8 probe** 13470-L11728			**Exon 4**			
463	**TPO probe** 13471-L11729		**Exon 14**				
472	**TSHR probe** 13474-L11731						**Exon 6**
481	Reference probe 09966-L10425	17q12					

* New in version B1 (from lot B1-0718 onwards).

¥ Changed in version B1 (from lot B1-0718 onwards). Small change in length, no change in sequence detected.

Table 2. P319-B1 probes arranged according to chromosomal location

Table 2a. *TPO*

Length (nt)	SALSA MLPA probe	*TPO* exon	Ligation site NM_000547.5	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*92-94 (exon 2)*		
130	10913-L11582	Exon 1	79 nt after exon 1	GAGTGGCTGTAA-TTTGGGCCATTA	19.9 kb
	No probe	Exon 2			
	No probe	Exon 3			
319	05845-L14485	Exon 4	352-353	AGCGGAGTGATT-GCCCGAGCAGCA	2.8 kb
409	11056-L15926	Exon 5	520-521	CCAAAATGCCCA-AACACTTGCCTG	17.5 kb
231 ¥	22021-L31280	Exon 6	44 nt after exon 6	GATTGGGTCCTG-TGATGCTGAGGG	2.3 kb
337	11049-L11718	Exon 7	769-770	GATGACCGCTAT-TCTGACCTCCTG	21.1 kb
283	11044-L11713	Exon 8	1010-1011	CGCTCTTTGGGA-ACCTGTCCACGG	0.0 kb
444	13472-L11732	Exon 8	1060-1059 reverse	GACGCGTCCAGG-AACGAGGTCAAC	7.4 kb
373	11052-L11721	Exon 9	1509-1510	CTATGAAGGCTA-TGACTCCACCGC	3.2 kb
426	11058-L15927	Exon 10	1725-1726	CCTTCTTGCAAG-ACCAGCCAAACT	5.9 kb
178	10972-L11643	Exon 11	46 nt before exon 11	TGGGCTGAACAA-AAGTTCAGTTCT	2.5 kb
301	11046-L11715	Exon 12	54 nt after exon 12	CTCATCAAACAA-AGCTTATCTTCC	0.3 kb
382	13469-L11723	Exon 13	43 nt before exon 13	TGTGGTTTTCTT-TTCTCGTAGTTT	7.5 kb
463	13471-L11729	Exon 14	2599-2600	GACGATGGGAGA-ACCTGCGTAGGT	12.9 kb
142	10920-L14478	Exon 15	2666-2667	CTGCTCTGCTGA-TCGGAGGCTTCG	23.7 kb
250	11040-L15610	Exon 16	2741-2742	CCACACTGCCCA-TCTCGGAGACAG	1.6 kb
436	13473-L11733	Exon 17	182 nt before exon 17	TCCTGCAAACAA-GCATTTGTCCGG	
		Stop Codon	*2891-2893 (exon 17)*		

¥ Changed in version B1 (from lot B1-0718 onwards). Small change in length, no change in sequence detected.

Note: The exon numbering used in this P319-B1 Thyroid product description is the exon numbering from the RefSeq transcript NM_000547.5. The exon numbering and NM sequence used is from 07/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2b. *PAX8*

Length (nt)	SALSA MLPA probe	*PAX8* exon	Ligation site NM_013953.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*167-169 (exon2)*		
202	10977-L11648	Exon 1	16-17	ACAAACTTCAGA-AGGAGGAGAGAC	0.1 kb
160	10931-L11600	Exon 1	24 nt after exon 1	GGGTTAGCTGGA-AGCTGGCTAGCA	0.4 kb
172	10971-L11642	Exon 2	3 nt after exon 2	TCAGATCTGGTA-AGAACGCGGTGT	33.5 kb
	No probe	Exon 3			
453	13470-L11728	Exon 4	273 nt before exon 4	TTGCAGATGCTA-GGACACAAGAGA	2.2 kb
400	11055-L11724	Exon 5	609-610	GGACAGCTGCGT-GGCCACCAAGTC	1.3 kb
	No probe	Exon 6			
346	11050-L14486	Exon 7	94 nt after exon 7	AAAGCAGCTGGA-AGTTGCATCAAT	14.3 kb
256	12810-L14483	Exon 8	1013-1014	ACAGGGCAGCTA-TGCCTCCTCTGC	7.0 kb
190	10974-L15609	Exon 9	1069-1070	CTGGCAATGCCT-ATGGCCACACCC	1.8 kb
220	10981-L14482	Exon 10	1389-1390	TGACCTTGGACA-AGGCCAAACTGT	
		Stop Codon	*1130-1132 (exon 9)*		

Note: The exon numbering used in this P319-B1 Thyroid product description is the exon numbering from the RefSeq transcript NM_013953.3. The exon numbering and NM sequence used is from 07/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2c. *FOXE1*

Length (nt)	SALSA MLPA probe	***FOXE1*** **exon**	Ligation site NM_004473.3	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*661-663 (exon 1)*		
267	11042-L16031	Exon 1	2118-2119	ACCAGGATCCAA-ATTGTGGGGAAT	0.5 kb
238	11038-L11706	Exon 1	2611-2612	GCGTCTAACCTA-AAGTCCCAGGAT	
		Stop Codon	*1780-1782 (exon 1)*		

Note: The exon numbering used in this P319-B1 Thyroid product description is the exon numbering from the RefSeq transcript NM_004473.3. The exon numbering and NM sequence used is from 06/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2d. *NKX2-1*

Length (nt)	SALSA MLPA probe	***NKX2-1*** **exon**	Ligation site NM_001079668.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*97-99 (exon 1)*		
136	10917-L14480	Exon 1	61-62	TCGCTCGCTCAT-TTGTTGGCGACT	0.6 kb
355	11051-L14487	Exon 2	188 nt before exon 2	GGGCTAAAACAA-ACGCGAGGCAGC	2.7 kb
208	10978-L11649	Exon 3	1712-1713	CTGGGCACACTC-TGCCAGCAAAGA	
		Stop Codon	*1300-1302 (exon 3)*		

Note: The exon numbering used in this P319-B1 Thyroid product description is the exon numbering from the RefSeq transcript NM_001079668.2. The exon numbering and NM sequence used is from 06/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

Table 2e. *TSHR*

Length (nt)	SALSA MLPA probe	***TSHR*** **exon**	Ligation site NM_000369.2	Partial sequence (24 nt adjacent to ligation site)	Distance to next probe
		Start Codon	*157-159 (exon 1)*		
154	10928-L11597	Exon 1	269-270	GGAGGACTTCAG-AGTCACCTGCAA	106.4 kb
244	11039-L11707	Exon 2	361-362	GAACTATTCCAA-GTCATGCATTTT	6.1 kb
274	11043-L11711	Exon 3	425-426	TGTGACTCTGCA-GCAGCTGGAATC	19.7 kb
310	12809-L14484	Exon 4	506-507	CTTAACTTACAT-AGACCCTGATGC	3.1 kb
184	10973-L11644	Exon 5	577-578	GACTTAAAATGT-TCCCTGACCTGA	1.5 kb
472	13474-L11731	Exon 6	665-666	AATCCCTGTGAA-TGCTTTTCAGGG	4.1 kb
196	10975-L11646	Exon 7	756-757	TTCAATGGGACA-AAGCTGGATGCT	11.7 kb
418	11057-L11726	Exon 8	821-822	AGATGCATTTGG-AGGAGTATACAG	31.3 kb
292	11045-L11714	Exon 9	936-937	ACCTGGACTCTT-AAGAAACTTCCA	4.7 kb
166	10934-L11604	Exon 10	2579-2580	ATGTTTCAATGT-TTCATGGGGCAA	
		Stop Codon	*2449-2451 (exon 10)*		

Note: The exon numbering used in this P319-B1 Thyroid product description is the exon numbering from the RefSeq transcript NM_000369.2, which is identical to the LRG_523 sequence. The exon numbering and NM sequence used is from 08/2018, but can be changed (e.g. by NCBI) after the release of the product description. Please notify us of any mistakes: info@mlpa.com.

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 P319 Thyroid

- Kumorowicz-Czoch et al (2015). Identification of deletions in children with congenital hypothyroidism and thyroid dysgenesis with the use of multiplex ligation-dependent probe amplification. *J Pediatr Endocrinol Metab.* 28(1-2):171-6.
- Teissier R et al (2012). Multiplex Ligation-Dependent Probe Amplification Improves the Detection Rate of NKX2.1 Mutations in Patients Affected by Brain-Lung-Thyroid Syndrome. *Horm Res Paediatr.* 77:146–151.

P319 Product history	
Version	*Modification*
B1	One probe for *PAX8* and one for *TSHR* have been removed, three reference probes have been replaced and several probe lengths have been adjusted.
A2	QDX2 control fragments included.
A1	First release.

Implemented changes in the product description

Version B1-01 – 27 September 2018 (01P)
- Product description restructured and adapted to a new template.
- Product description adapted to a new product version.
- Textual changes on page 1 and small changes in Table 1 and Table 2).

Version 07 – 03 March 2017 (55)
- Warning added in Table 1 and Table 2, 267 nt probe 11042-L16031.
- Minor textual changes.

Version 06 - 09 December 2015 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Exon numbering of the *PAX8* and *TSHR* gene has been changed on page 3 and 4.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 05 (48)- 06 August 2015
- Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

Version 04 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

Version 03 (48)
- Warning added below Table 2 that the exon numbering used is different from the NCBI exon numbering in the NM_ reference sequence.
- Remark on RefSeqGene standard and transcript variant added below Table 2.
- Various minor textual changes.

Version 02 (46)
- New references added on page 1.
- Various minor textual changes.
- Various minor layout changes.
- Warning added in Table 1, 124 nt probe 09176-L09350 and 500 nt probe 13725-L15677.

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