Product Description
SALSA® MLPA® Probemix P319-B2 Thyroid

To be used with the MLPA General Protocol.

Version B2
As compared to version B1, five reference probes have been replaced and one reference probe has been added. In addition, many probes have been adjusted in probe length. For complete product history see page 9.

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 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 P319 Thyroid is a **research use only (RUO)** assay for the detection of deletions or duplications in *TPO*, *PAX8*, *FOXE1*, *NKX2-1* and *TSHR* genes, which are associated with thyroid dysgenesis.

Thyroid dysgenesis (TD) accounts for most cases of congenital hypothyroidism. TD is generally a sporadic disease and in most cases the cause is unknown. Occasional familial occurrence and a difference in prevalence of TD between males vs. females, between ethnicities, and in patients with Down syndrome all suggest genetic factors may play a role in some patients. Mutations in genes playing a role during thyroid morphogenesis (*TPO*, *PAX8*, *FOXE1*, *NKX2-1*, *TSHR* amongst others) have been reported.

The *TPO* gene (17 exons) spans ~129 kb of genomic DNA and is located on 2p25.3, ~1.4 Mb from the p-telomere. The *PAX8* gene (10 exons) spans ~63 kb of genomic DNA and is located on 2q13, ~114 Mb from the p-telomere. The *FOXE1* gene (1 exon) spans ~4 kb of genomic DNA and is located on 9q22.33, ~100 Mb from the p-telomere. The *NKX2-1* gene (3 exons) spans ~4 kb of genomic DNA and is located on 14q13.3, ~36 Mb from the p-telomere. The *TSHR* gene (10 exons) spans ~191 kb of genomic DNA and is located on 14q31.1, ~81 Mb from the p-telomere.

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/

Exon numbering
The exon numbering used in this P319-B2 Thyroid product description is the exon numbering from:

Gene	NG or LRG sequence
TPO	NG_011581.1
PAX8	NG_012384.1
FOXE1	NG_011979.1
NKX2-1	NG_013365.1
TSHR	LRG_523

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 NG or 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 P319-B2 Thyroid contains 50 MLPA probes with amplification products between 127 and 481 nucleotides (nt). This includes 16 probes for the *TPO* gene (one probe for each exon with the exception of exon 2 and 3, and two probes for exon 8), nine probes for the *PAX8* gene (one probe for each exon with the exception of exon 3 and 6, and two probes for exon 1), two probes for the *FOXE1* gene, three probes for the *NKX2-1* gene (one probe for each exon) and ten probes for the *TSHR* gene (one probe for each exon). In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal indicates incomplete denaturation)
92	Benchmark fragment
100	X-fragment (X chromosome specific)
105	Y-fragment (Y chromosome specific)

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

MLPA technique validation
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

Required specimens
Extracted DNA, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of thyroid abnormalities. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA10401, NA13590, NA22765, NA06801 and NA16593 from the Coriell Institute have been tested with this P319-B2 probemix at MRC Holland and can be used as positive control samples to detect the copy number alterations described in the table below. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Altered target genes in P319-B2	Expected copy number alteration
NA10401	Coriell Institute	*TPO* and *PAX8*	Heterozygous duplication
NA13590	Coriell Institute	*PAX8*	Homozygous duplication
NA22765	Coriell Institute	*NKX2-1*	Heterozygous deletion
NA06801	Coriell Institute	*NKX2-1*	Heterozygous duplication
NA16593	Coriell Institute	*TSHR*	Heterozygous deletion in exon 1-8

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 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

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

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.

- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *TPO*, *PAX8*, *FOXE1*, *NKX2*-1 and *TSHR* genes are small (point) mutations, most 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. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

Confirmation of results

Copy number changes detected by only a single probe always require confirmation by another method. An apparent deletion detected by a single probe can be due to e.g. a mutation/polymorphism that prevents ligation or destabilises the binding of probe oligonucleotides to the DNA sample. Sequence analysis can establish whether mutations or polymorphisms are present in the probe target sequence. The finding of a heterozygous mutation or polymorphism indicates that two different alleles of the sequence are present in the sample DNA and that a false positive MLPA result was obtained.

Copy number changes detected by more than one consecutive probe should be confirmed by another independent technique such as long range PCR, qPCR, array CGH or Southern blotting, whenever possible. Deletions/duplications of more than 50 kb in length can often be confirmed by FISH.

***TPO*, *PAX8*, *FOXE1*, *NKX2-1* and *TSHR* mutation databases**

https://databases.lovd.nl/shared/genes/TPO, https://databases.lovd.nl/shared/genes/PAX8, https://databases.lovd.nl/shared/genes/FOXE1, https://databases.lovd.nl/shared/genes/NKX2-1 and https://databases.lovd.nl/shared/genes/TSHR. We strongly encourage users to deposit positive results in the

Leiden Open Variation 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 SNVs and unusual results (e.g., a duplication of *TPO* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P319-B2 Thyroid

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		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	5q					
133 ¥	**TPO probe** 10913-L32842		**Exon 1**				
139 ¥	**NKX2-1 probe** 10917-L32865					**Exon 1**	
145 ¥	**TPO probe** 10920-L32874		**Exon 15**				
149 *	Reference probe 19618-L32875	10p					
156 ¥	**TSHR probe** 10928-L32876						**Exon 1**
161 ¥	**PAX8 probe** 10931-L32843			**Exon 1**			
166	**TSHR probe** 10934-L11604						**Exon 10**
174 ¥	**PAX8 probe** 10971-L32845			**Exon 2**			
179 ¥	**TPO probe** 10972-L32846		**Exon 11**				
186 ¥	**TSHR probe** 10973-L32847						**Exon 5**
190 ¥	**PAX8 probe** 10974-L32867			**Exon 9**			
196	**TSHR probe** 10975-L11646						**Exon 7**
202	**PAX8 probe** 10977-L11648			**Exon 1**			
208	**NKX2-1 probe** 10978-L11649					**Exon 3**	
215 *	Reference probe 03826-L22080	3q					
220 ¥	**PAX8 probe** 22373-L32848			**Exon 10**			
226	Reference probe 10672-L31279	6p					
232 ¥	**TPO probe** 22021-L32868		**Exon 6**				
238	**FOXE1 probe** 11038-L11706				**Exon 1**		
244	**TSHR probe** 11039-L11707						**Exon 2**
250	**TPO probe** 11040-L15610		**Exon 16**				
256 *	Reference probe 22330-L31452	17q					
261 ¥	**PAX8 probe** 12810-L32840			**Exon 8**			
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**
300 *	Reference probe 21328-L29734	7q					
308 ¥	**TPO probe** 11046-L32849		**Exon 12**				
319 ¥	**TSHR probe** 12809-L32850						**Exon 4**
325 ¥	**TPO probe** 05845-L32851		**Exon 4**				
331 *	Reference probe 08905-L24614	11p					
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	15q					
373	**TPO probe** 11052-L11721		**Exon 9**				
382	**TPO probe** 13469-L11723		**Exon 13**				
391	Reference probe 12762-L13878	4q					
406 ¥	**PAX8 probe** 11055-L32852			**Exon 5**			
415 ¥	**TPO probe** 11056-L32853		**Exon 5**				
422 ¥	**TSHR probe** 11057-L32854						**Exon 8**
427 ¥	**TPO probe** 11058-L32869		**Exon 10**				
436 ¥	**TPO probe** 13473-L32872		**Exon 17**				
444	**TPO probe** 13472-L11732		**Exon 8**				
454 ¥	**PAX8 probe** 13470-L32856			**Exon 4**			
465 ¥	**TPO probe** 13471-L32857		**Exon 14**				
474 ¥	**TSHR probe** 13474-L32858						**Exon 6**
481 *	Reference probe 15490-L17330	18q					

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

* New in version B2.
¥ Changed in version B2. Minor alteration, no change in sequence detected.

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

Table 2. P319-B2 probes arranged according to chromosomal location

Table 2a. *TPO* gene

Length (nt)	SALSA MLPA probe	*TPO* exon[a]	Ligation site NM_000547.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*90-92 (Exon 2)*		
133	10913-L32842	Exon 1	80 nt after exon 1	GAGTGGCTGTAA-TTTGGGCCATTA	19.9 kb
	No probe	Exon 2			
	No probe	Exon 3			
325	05845-L32851	Exon 4	350-351	AGCGGAGTGATT-GCCCGAGCAGCA	2.8 kb
415	11056-L32853	Exon 5	518-519	CCAAAATGCCCA-AACACTTGCCTG	17.5 kb
232	22021-L32868	Exon 6	45 nt after exon 6	GATTGGGTCCTG-TGATGCTGAGGG	2.3 kb
337	11049-L11718	Exon 7	767-768	GATGACCGCTAT-TCTGACCTCCTG	21.1 kb
283	11044-L11713	Exon 8	1008-1009	CGCTCTTTGGGA-ACCTGTCCACGG	0.0 kb
444	13472-L11732	Exon 8	1058-1057 *reverse*	GACGCGTCCAGG-AACGAGGTCAAC	7.4 kb
373	11052-L11721	Exon 9	1507-1508	CTATGAAGGCTA-TGACTCCACCGC	3.2 kb
427	11058-L32869	Exon 10	1723-1724	CCTTCTTGCAAG-ACCAGCCAAACT	5.9 kb
179	10972-L32846	Exon 11	46 nt before exon 11	TGGGCTGAACAA-AAGTTCAGTTCT	2.5 kb
308	11046-L32849	Exon 12	55 nt after exon 12	CTCATCAAACAA-AGCTTATCTTCC	0.3 kb
382	13469-L11723	Exon 13	43 nt before exon 13	TGTGGTTTTCTT-TTCTCGTAGTTT	7.5 kb
465	13471-L32857	Exon 14	2597-2598	GACGATGGGAGA-ACCTGCGTAGGT	12.9 kb
145	10920-L32874	Exon 15	2664-2665	CTGCTCTGCTGA-TCGGAGGCTTCG	23.7 kb
250	11040-L15610	Exon 16	2739-2740	CCACACTGCCCA-TCTCGGAGACAG	1.6 kb
436	13473-L32872	Exon 17	182 nt before exon 17	TCCTGCAAACAA-GCATTTGTCCGG	
		stop codon	*2889-2891 (Exon 17)*		

Table 2b. *PAX8* gene

Length (nt)	SALSA MLPA probe	*PAX8* exon[a]	Ligation site NM_013953.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*167-169 (Exon 2)*		
202	10977-L11648	Exon 1	16-17	ACAAACTTCAGA-AGGAGGAGAGAC	0.1 kb
161	10931-L32843	Exon 1	25 nt after exon 1	GGGTTAGCTGGA-AGCTGGCTAGCA	0.4 kb
174	10971-L32845	Exon 2	3 nt after exon 2	TCAGATCTGGTA-AGAACGCGGTGT	33.5 kb
	No probe	Exon 3			
454	13470-L32856	Exon 4	273 nt before exon 4	TTGCAGATGCTA-GGACACAAGAGA	2.2 kb
406	11055-L32852	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
261	12810-L32840	Exon 8	1013-1014	ACAGGGCAGCTA-TGCCTCCTCTGC	7.0 kb
190	10974-L32867	Exon 9	1069-1070	CTGGCAATGCCT-ATGGCCACACCC	1.8 kb
220	22373-L32848	Exon 10	1389-1390	TGACCTTGGACA-AGGCCAAACTGT	
		stop codon	*1130-1132 (Exon 9)*		

Table 2c. *FOXE1* gene

Length (nt)	SALSA MLPA probe	*FOXE1* exon[a]	Ligation site NM_004473.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*690-692 (Exon 1)*		
267	11042-L16031	Exon 1	2147-2148	ACCAGGATCCAA-ATTGTGGGGAAT	0.5 kb
238	11038-L11706	Exon 1	2640-2641	GCGTCTAACCTA-AAGTCCCAGGAT	
		stop codon	*1809-1811 (Exon 1)*		

Table 2d. *NKX2-1* gene

Length (nt)	SALSA MLPA probe	*NKX2-1* exon[a]	Ligation site NM_001079668.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*104-106 (Exon 1)*		
139	10917-L32865	Exon 1	68-69	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	1719-1720	CTGGGCACACTC-TGCCAGCAAAGA	
		stop codon	*1307-1309 (Exon 3)*		

Table 2e. *TSHR* gene

Length (nt)	SALSA MLPA probe	*TSHR* exon[a]	Ligation site NM_000369.5	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*61-63 (Exon 1)*		
156	10928-L32876	Exon 1	173-174	GGAGGACTTCAG-AGTCACCTGCAA	106.4 kb
244	11039-L11707	Exon 2	265-266	GAACTATTCCAA-GTCATGCATTTT	6.1 kb
274	11043-L11711	Exon 3	329-330	TGTGACTCTGCA-GCAGCTGGAATC	19.7 kb
319	12809-L32850	Exon 4	410-411	CTTAACTTACAT-AGACCCTGATGC	3.1 kb
186	10973-L32847	Exon 5	481-482	GACTTAAAATGT-TCCCTGACCTGA	1.5 kb
474	13474-L32858	Exon 6	569-570	AATCCCTGTGAA-TGCTTTTCAGGG	4.1 kb
196	10975-L11646	Exon 7	660-661	TTCAATGGGACA-AAGCTGGATGCT	11.7 kb
422	11057-L32854	Exon 8	725-726	AGATGCATTTGG-AGGAGTATACAG	31.3 kb
292	11045-L11714	Exon 9	840-841	ACCTGGACTCTT-AAGAAACTTCCA	4.7 kb
166	10934-L11604	Exon 10	2483-2484	ATGTTTCAATGT-TTCATGGGGCAA	
		stop codon	*2353-2355 (Exon 10)*		

[a] See section Exon numbering on page 2 for more information.
[b] Only partial probe sequences are shown. Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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

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

- Alcántara-Ortigoza MA et al. (2021). Further evidence that defects in main thyroid dysgenesis-related genes are an uncommon etiology for primary congenital hypothyroidism in mexican patients: report of rare variants in FOXE1, NKX2-5 and TSHR. *Children (Basel)*. 8:457.

- 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*. 28:171-6.
- Makretskaya N et al. (2018). High frequency of mutations in'dyshormonogenesis genes' in severe congenital hypothyroidism. *PLoS One*. 13:e0204323.
- 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.
- Villafuerte B et al. (2018). The brain-lung-thyroid syndrome (BLTS): a novel deletion in chromosome 14q13. 2-q21.1 expands the phenotype to humoral immunodeficiency. *Eur J Med Genet*. 61:393-398.

P319 product history	
Version	*Modification*
B2	Five reference probes have been replaced and one reference probe has been added. In addition, many probes have been adjusted in probe length.
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 B2-01 – 5 July 2022 (04P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2). - Ligation sites of the probes targeting the *TPO*, *PAX8*, *FOXE1*, *NKX2-1* and *TSHR* genes updated according to new version of the NM_ reference sequence. *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).

More information: www.mrcholland.com; www.mrcholland.eu	
	MRC Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mrcholland.com (information & technical questions) order@mrcholland.com (orders)
Phone	+31 888 657 200