Product Description
SALSA® MLPA® Probemix P431-B1 FOXF1

To be used with the MLPA General Protocol.

Version B1
For complete product history see page 7.

Catalogue numbers:
- **P431-025R:** SALSA MLPA Probemix P431 FOXF1, 25 reactions.
- **P431-050R:** SALSA MLPA Probemix P431 FOXF1, 50 reactions.
- **P431-100R:** SALSA MLPA Probemix P431 FOXF1, 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 P431 FOXF1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *FOXF1* gene, the 16q24.1 chromosomal region, and the *MYCN* gene, which are associated with Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV) and Feingold syndrome (FS) respectively.

ACD/MPV is a rare, neonatal and lethal development disorder of the lungs. In patients with ACD/MPV, Stankiewicz et al. identified four different heterozygous mutations in the candidate *FOXF1* gene and overlapping micro-deletions encompassing the FOX transcription factor gene cluster on chromosome 16q24.1. (Stankiewicz P et al. 2009, Am J Hum Genet. 84:780-91). In addition, deletions in either a shared deletion region (SDR) of 75 kb, located 257 kb upstream of *FOXF1*, and a 0.8 kb deletion within the 1.4 kb intron 1 of *FOXF1* have been found (Szafranski P et al. 2013, Genome Res. 23:23-33; Szafranski P et al. 2013, Hum Mut. 11:1467-71).

FS is a rare autosomal dominant inherited condition that is characterised by microcephaly, limb malformations, esophageal atresia, and other malformations. Defects (haploinsufficiency, mutations, (micro)deletions) in the *MYCN* proto-oncogene on chromosome 2p24.3 is the main cause of FS. The *MYCN* gene is a member of the MYC family and encodes the transcriptional regulator N-myc regulating many target genes involved in the cell cycle.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK7050/.

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 *FOXF1*, *MYCN, FOXL1*, and *FOXC2* exon numbering used in this P431-B1 FOXF1 product description is the exon numbering from the NG_016273.1, NG_007457.2, LRG_709, and LRG_1292 sequences respectively. 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/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 P431-B1 FOXF1 contains 38 MLPA probes with amplification products between 130 and 454 nucleotides (nt). This includes 25 probes for the *FOX* gene cluster region: ten probes for the shared deletion region (SDR), which includes four probes for the regulatory fragment 1a (RF1a), two probes upstream of *FOXF1* gene, two probes for exon 1 and 2, four probes for intron 1 of the *FOXF1* gene, two probes for exon 1 of the *FOXC2* gene, and three probes for exon 1 of the *FOXL1* gene. Furthermore, this probemix includes five probes for the *MYCN* gene, two probes for exon 1 and 3 and one probe for exon 2. In addition, eight 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 Alveolar Capillary Dysplasia with Misalignment of Pulmonary Veins (ACD/MPV) and Feingold syndrome (FS). 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 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.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 or in or near the *FOXF1*, *FOXC2* and *MYCN* 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.
- 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 *MYCN* and *FOXF1* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P431 FOXF1.
- 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.

***FOXF1* and *MYCN* mutation database**

https://databases.lovd.nl/shared/genes. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 *MYCN* exons 1 and 3 but not exon 2) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P431-B1 FOXF1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]				
		Reference	*MYCN*	*FOX* gene cluster		
				FOXF1	*FOXC2*	*FOXL1*
64-105	Control fragments – see table in probemix content section for more information					
130	Reference probe 11230-L11913	8q				
139 +	**FOXF1 probe** 21475-L31130			**SDR**		
145 Ø	**FOXF1 probe** 21834-L30561			**Intron 1**		
150 ๑	**FOXF1 probe** 19248-L31131			**RF1a**		
154 +	**FOXF1 probe** 19249-L25354			**SDR**		
166 Ø	**FOXF1 probe** 21835-L30562			**Intron 1**		
171 « Δ	**MYCN probe** 18793-L24223		**Exon 1**			
179	Reference probe 10509-L25743	7q				
190 Ø	**FOXF1 probe** 21836-L31132			**Intron 1**		
196 «	**FOXC2 probe** 19104-L25009				**Exon 1**	
200 «	**FOXF1 probe** 18583-L25946			**Exon 1**		
208 «	**MYCN probe** 17473-L25814		**Exon 3**			
214	Reference probe 07733-L07423	20q				
220 «	**FOXF1 probe** 18580-L18597			**Upstream**		
226 Ø	**FOXF1 probe** 21837-L30564			**Intron 1**		
235 «	**FOXF1 probe** 21474-L25815			**Exon 2**		
255 «	**FOXF1 probe** 18581-L18633			**Upstream**		
265	Reference probe 19015-L25096	21q				
271 «	**FOXF1 probe** 18585-L25816			**Exon 2**		
278	**FOXL1 probe** 21473-L25011					**Exon 1**
289 «	**FOXF1 probe** 18582-L18377			**Exon 1**		
300 «	**MYCN probe** 18792-L21412		**Exon 3**			
310 «	**MYCN probe** 18794-L24224		**Exon 1**			
319	Reference probe 03918-L03373	15q				
328 «	**FOXC2 probe** 19107-L25012				**Exon 1**	
337	**FOXL1 probe** 19108-L25013					**Exon 1**
346 +	**FOXF1 probe** 19251-L25356			**SDR**		
355	Reference probe 15081-L16844	4q				
364 ๑	**FOXF1 probe** 19252-L25357			**RF1a**		
373 +	**FOXF1 probe** 19253-L25358			**SDR**		
382	Reference probe 21221-L29596	9p				
391 +	**FOXF1 probe** 19254-L25359			**SDR**		
400 ๑	**FOXF1 probe** 19255-L25360			**RF1a**		
418 ๑	**FOXF1 probe** 22353-L25812			**RF1a**		
427 +	**FOXF1 probe** 22354-L25355			**SDR**		
436	**FOXL1 probe** 22356-L25008					**Exon 1**
445 «	**MYCN probe** 22357-L21406		**Exon 2**			
454	Reference probe 08579-L08580	17q				

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

« 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.
+ These probes are designed specific for the shared deletion region (SDR) of 75 kb, located 257 kb upstream of the *FOXF1* gene (Szafranski P et al. 2013, *Genome Res*. 23:23-33).
๑ These probes are designed specific for the regulatory fragment 1a (RF1a) of 1505 bp, within the SDR, 257 kb upstream of the *FOXF1* gene (Szafranski P et al. 2013, *Genome Res*. 23:23-33).
Ø These probes are located in Intron 1 of *FOXF1*, deletions of 0.8 kb within the 1.4 kb intron have been found (Szafranski P et al. 2013, *Hum Mut*. 11:1467-71).
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution

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. P431-B1 probes arranged according to chromosomal location

Table 2a. *MYCN*

Length (nt)	SALSA MLPA probe	*MYCN* exon[a]	Ligation site NM_005378.6	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*312-314 (Exon 2)*		
310 «	18794-L24224	Exon 1	19-20	GACAGTCATCTG-TCTGGACGCGCT	0.1 kb
171 « Δ	18793-L24223	Exon 1	162-163	CACCCGCGCAGA-ATCGCCTCCGGA	1.5 kb
445 «	22357-L21406	Exon 2	470-471	TGGAAGAAGTTT-GAGCTGCTGCCC	3.4 kb
300 «	18792-L21412	Exon 3	1200-1201	CTGTCACCACAT-TCACCATCACTG	0.3 kb
208 «	17473-L25814	Exon 3	1452-1453	CGGAGGACAGTG-AGCGTCGCAGAA	
		stop codon	*1704-1706 (Exon 3)*		

Table 2b. *FOX* gene cluster

Length (nt)	SALSA MLPA probe	Location/ exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
SDR upstream of the *FOXF1* gene (RP11-514D23 (AC040170.9))					
154 +	19249-L25354	SDR	149475-149474	ATGCTGCAGTAT-TGCCTAACCAGA	7.0 kb
139 +	21475-L31130	SDR	142800-142799	GTGGACCTGCAC-AGGCTTTGTGTT	7.8 kb
391 +	19254-L25359	SDR	134601-134600	ATGGGGATTCTG-TCTACAGTGACA	1.7 kb
150 ◎	19248-L31131	RF1a	132900-132899	TATGCCAGGCAT-AGGTGTCAGGGA	0.4 kb
418 ◎	22353-L25812	RF1a	132519-132518	AGAGAGCAGGGC-AACTGCACTAAG	0.5 kb
364 ◎	19252-L25357	RF1a	132008-132007	CAGGCGTTTATG-CCGTTATGAATG	0.3 kb
400 ◎	19255-L25360	RF1a	131715-131714	TGACTGCTAATG-GATGACCACCCA	4.4 kb
373 +	19253-L25358	SDR	127285-127284	ACCGGTGGCTGA-CTGCTTTTCCTT	23.0 kb
346 +	19251-L25356	SDR	104291-104290	AACTCACCAAGT-GCATTGAAAGGG	20.9 kb
427 +	22354-L25355	SDR	83364-83363	GCCAAGCTCAGA-ATCTCACAGGAG	262.0 kb
***FOXF1* gene (NM_001451.3)**					
		start codon	*44-46 (Exon 1)*		
220 «	18580-L18597	Upstream	917 nt before exon 1	GGCGAGTCCGAA-AAATCCCGGGAG	0.4 kb
255 «	18581-L18633	Upstream	476 nt before exon 1	ACGCAGCCGAGC-GGAGATGGAGTG	0.6 kb
289 «	18582-L18377	Exon 1	158-159	AGGCCAAGAAGA-CCAACGCCGGCA	0.2 kb
200 # «	18583-L25946	Exon 1	370-371	TTCATCAAGCTA-CCCAAGGGCCTT	0.8 kb
226 Ø	21837-L30564	Intron 1	190 nt after exon 1, reverse	GATTGGCCCAAA-CTAGGGTCAAGA	0.1 kb
190 Ø	21836-L31132	Intron 1	338 nt after exon 1, reverse	CCATCCCAGGAT-CTTTCCAAGCTG	0.4 kb
166 Ø	21835-L30562	Intron 1	682 nt before exon 2, reverse	TGCTTCTGCATG-TTCGGGGTATTG	0.1 kb
145 Ø	21834-L30561	Intron 1	569 nt before exon 2	GAGCTTGCCAGG-GCCAGTCCAGGG	0.6 kb
235 «	21474-L25815	Exon 2	1079-1080	AGGAGTTTGTCT-TCTCTTTCAACG	0.6 kb
271 «	18585-L25816	Exon 2	1703-1704	AGCCGTCTTTTG-CAGGGAGCGGGA	53.8 kb
		stop codon	*1181-1183 (Exon 2)*		
***FOXC2* (NM_005251.3)**					
		start codon	*508-510 (Exon 1)*		
328 «	19107-L25012	Exon 1	565-566	TGCCCTACCTGA-GCGAGCAGAATT	1.3 kb
196 «	19104-L25009	Exon 1	1899-1900	ATTGAGAACTCG-ACCCTCGGGGAG	10.0 kb
		stop codon	*2011-2013 (Exon 1)*		
***FOXL1* (NM_005250.3)**					
		start codon	*176-178 (Exon 1)*		
278	21473-L25011	Exon 1	179-180	CGCTTGCCATGA-GTCACCTCTTCG	1.3 kb

Length (nt)	SALSA MLPA probe	Location/ exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
436	22356-L25008	Exon 1	1505-1506	TTCCTTGACGTT-TGACCTGTCTAA	0.9 kb
337	19108-L25013	Exon 1	2400-2401	TTTGATGGCAGG-AATCTCCCAGAC	
		stop codon	*1211-1213 (Exon 1)*		

[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.

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.
« 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.
+ These probes are designed specific for the shared deletion region (SDR) of 75 kb, located 257 kb upstream of the *FOXF1* gene (Szafranski P et al. 2013, *Genome Res*. 23:23-33).
⊙ These probes are designed specific for the regulatory fragment 1a (RF1a) of 1505 bp, within the SDR, 257 kb upstream of the *FOXF1* gene (Szafranski P et al. 2013, *Genome Res*. 23:23-33).
Ø These probes are located in Intron 1 of *FOXF1*, deletions of 0.8 kb within the 1.4 kb intron have been found (Szafranski P et al. 2013, *Hum Mut*. 11:1467-71).
Δ More variable. This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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.
- Stankiewicz P et al. (2009). Genomic and genic deletions of the FOX gene cluster on 16q24. 1 and inactivating mutations of FOXF1 cause alveolar capillary dysplasia and other malformations. *Am J Hum Genet*. 84(6), 780-791.
- Szafranski P et al. (2013). Small noncoding differentially methylated copy-number variants, including lncRNA genes, cause a lethal lung developmental disorder. *Genome Res*, 23(1), 23-33.
- Szafranski P et al. (2013). Novel FOXF1 deep intronic deletion causes lethal lung developmental disorder, alveolar capillary dysplasia with misalignment of pulmonary veins. *Hum Mut*. 11:1467-71
- 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 P431 FOXF1

- Neuhäuser CA et al. (2021). Successful Management of an Infant with Atypical Presentation of Alveolar Capillary Dysplasia with Misalignment of the Pulmonary Veins. *J Pediatr Intensive Care*, 10(03), 228-231.
- Onda T et al. (2021). Incidence of alveolar capillary dysplasia with misalignment of pulmonary veins in infants with unexplained severe pulmonary hypertension: The roles of clinical, pathological, and genetic testing. *Early Hum Dev*, 155, 105323.

P431 product history	
Version	*Modification*
B1	Four *FOXF1* intron 1 probes have been included. Six reference probes have been replaced and five removed. In addition multiple probes have been adjusted in probe length.
A1	First release.

Implemented changes in the product description
Version B1-02 – 23 November 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *FOXF1*, *FOXL1* genes have been updated according to new version of the NM_ reference sequence. - Warning removed for 328 nt probe in Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Warning added to Table 1 and Table 2 on sensitivity of the 171 nt probe to certain experimental variations. *Version B1-01 — 29 August 2019 (02P)* - 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 *FOXC2* and *MYCN* genes have been updated according to new version of the NM_ reference sequence. - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.

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