Product Description
SALSA® MLPA® Probemix P476-A1 ZNRF3

To be used with the MLPA General Protocol.

Version A1
For complete product history see page 8.

Catalogue numbers:
- **P476-025R:** SALSA MLPA Probemix P476 ZNRF3, 25 reactions.
- **P476-050R:** SALSA MLPA Probemix P476 ZNRF3, 50 reactions.
- **P476-100R:** SALSA MLPA Probemix P476 ZNRF3, 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 P476 ZNRF3 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *ZNRF3* gene.

ZNRF3 is an E3 ubiquitin-protein ligase that acts as a negative feedback regulator of Wnt signalling (Hao et al. 2012). Three independent studies show homozygous deletions of the *ZNRF3* gene in 10 to 16% of adrenocortical carcinoma cases (Assié et al. 2014; Juhlin et al. 2015; Zheng et al. 2016). Moreover, in 51% of microsatellite stable colorectal cancers deletion events at the *ZNRF3* locus are detected (Bond et al. 2016). Deletions of the *ZNRF3* gene are also found in osteoblastoma and prostate cancer (Nord et al. 2013; Robinson et al. 2015, Fraser et al. 2021).

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 *ZNRF3* exon numbering used in this P476-A1 ZNRF3 product description is the exon numbering from the RefSeq transcript NM_001206998.2 and NM_032173.4. 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 P476-A1 ZNRF3 contains 42 MLPA probes with amplification products between 130 and 433 nucleotides (nt). This includes 15 probes for the *ZNRF3* gene, plus four flanking probes for upstream genes and five flanking probes for downstream genes. In addition, 18 reference probes are included that target relatively copy number stable regions in various cancer types including adrenocortical carcinoma,

colorectal cancer and prostate adenocarcinoma). Complete probe sequences and the identity of the genes detected by the reference probes are available in Table 3 and online (www.mrcholland.com).

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

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

MLPA technique
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com). More information on the use of MLPA in tumour applications can be found in Hömig-Hölzel and Savola (2012).

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

Required specimens
Extracted DNA, which includes DNA derived from paraffin-embedded tissues, free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol. More information on the use of FFPE tissue samples for MLPA can be found in Atanesyan et al. (2017).

Reference samples
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different healthy individuals without a history of cancer. 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 NA02325 and NA07106 from the Coriell Institute have been tested with this P476-A1 probemix at MRC Holland and can be used as positive control samples to detect a heterozygous duplication of the *ZNRF3* gene. 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. When this criterion is fulfilled, the following cut-off values for the final ratio (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.

Please note that these above mentioned final ratios are only valid for germline testing. Final ratios are affected both by percentage of tumour cells and by possible subclonality.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in subclonal cases.
- 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.

P476 specific note

- In samples from tumour tissues, reference probes are more prone to have deviating copy number results as compared to blood derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.
- Use of FFPE or fresh/frozen tissues can result in low quality of the extracted DNA due to sample fixation and storage conditions. This might result in higher probe standard deviations. Warnings during the Fragment Analysis using Coffalyser.Net will indicate that the MLPA experiment was not optimal on the specific sample(s) used. For more information on the use of FFPE tissues with MLPA, please refer to Atanesyan et al. 2017.

Limitations of the procedure

- In most populations, most genetic alterations in *ZNRF3* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P476 ZNRF3.
- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.
- MLPA analysis on tumour samples provides information on the *average* situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample, especially in tumour types with more chaotic karyotypes like adrenocortical carcinoma.

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.

COSMIC mutation database

http://cancer.sanger.ac.uk/cosmic. We strongly encourage users to deposit positive results in the COSMIC mutation database. Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report false positive results due to SNVs and unusual results (e.g., a duplication of *ZNRF3* exons 6 and 8 but not exon 7) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P476-A1 ZNRF3

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	*ZNRF3* (22q12.1)
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 19551-L26105	2p13	
136	Reference probe 11367-L12092	17q21	
142	**ZNRF3 probe** 21620-L30236		**Exon 5**
148	**ZNRF3 probe** 21621-L30237		**Exon 3**
154	Reference probe 13816-L15310	2q13	
160	**ZNRF3 probe** 21622-L30238		**Exon 9**
165 ¬	**NF2 probe** 21640-L22648		22q12.2; downstream
172	Reference probe 21193-L29851	6p12	
178	**ZNRF3 probe** 21623-L30684		**Exon 2**
184	**ZNRF3 probe** 21624-L30240		**Exon 9**
190	Reference probe 12409-L29450	10q21	
195 « ¬	**C22orf31 probe** 21625-L30241		22q12.1; downstream
202 «	**ZNRF3 probe** 21626-L30242		**Exon 1**
208	Reference probe 21639-L15999	2q32	
214	**ZNRF3 probe** 21627-L30243		**Exon 4**
220 ¬	**HSCB probe** 06821-L30685		22q12.1; upstream
226	Reference probe 08064-L30628	9p13	
232	**ZNRF3 probe** 21628-L30244		**Exon 7**
238	**ZNRF3 probe** 21629-L30245		**Exon 2**
244	Reference probe 21641-L30683	6q16	
250 « ¬	**CCDC117 probe** 21630-L30246		22q12.1; upstream
256 +	**ZNRF3 probe** 21631-L30247		**Intron 3**
265	**ZNRF3 probe** 21632-L30248		**Exon 10**
274	Reference probe 14827-L30626	3q11	
284	Reference probe 06725-L06313	15q24	
292 ¬	**AP1B1 probe** 15529-L17384		22q12.2; downstream
301 ¬	**CHEK2 probe** 06626-L30686		22q12.1; upstream
310 +	**ZNRF3 probe** 21633-L30249		**Intron 3**
319	Reference probe 15385-L17792	3p22	
328	**ZNRF3 probe** 21634-L30250		**Exon 8**
340	Reference probe 21099-L30929	11p15	
355 ¬	**KREMEN1 probe** 21635-L30251		22q12.1; downstream
364	**ZNRF3 probe** 21636-L30252		**Exon 6**
372	Reference probe 05953-L28763	2p22	
377	Reference probe 20767-L31004	1q24	
382	**ZNRF3 probe** 21637-L30253		61.6 kb upstream
391 « ¬	**EMID1 probe** 21638-L30254		22q12.2; downstream
400	Reference probe 01237-L00568	10p14	
409	Reference probe 14839-L30627	1p34	
418 ¬	**XBP1 probe** 21642-L30688		22q12.1; upstream
427	Reference probe 20872-L28890	1q42	
433	Reference probe 05290-L04670	14q22	

[a] See section Exon numbering on page 1 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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
+ Probe placed in *ZNRF3-AS1*, an antisense gene located in intron 3 of *ZNRF3*.

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. P476-A1 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Gene / exon[a]	Location / ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
Flanking probes upstream of *ZNRF3*.					
301 ¬	06626-L30686	*CHEK2*	22q12.1	AACCACTGCTGA-AAAGAACAGATA	18.7 kb
220 ¬	06821-L30685	*HSCB*	22q12.1	CCAGCAACTGCA-GCGTCTTGTCCA	37.0 kb
250 « ¬	21630-L30246	*CCDC117*	22q12.1	ATCCAGTCATTA-GGACCAGCACAG	14.7 kb
418 ¬ #	21642-L30688	*XBP1*	22q12.1	TGGACCCAGTCA-TGTTCTTCAAAT	26.3 kb
ZNRF3, at 22q12.1. Indicated ligation sites are in NM_001206998.2, unless otherwise specified.					
382	21637-L30253	Upstream	61,6 kb before exon 1	CAGTGTGCCTTC-AGCCAAAAGCAG	62.0 kb
		start codon	*196-198 (ex 1)*		
202 «	21626-L30242	Exon 1	397-398	TGCTGTTCGAGT-CGAGCCCAAGCG	33.4 kb
178	21623-L30684	Exon 2	NM_032173.4; 2-3	AGCCATCTGTGC-ACAGAAGTTTGC	0.1 kb
238	21629-L30245	Exon 2	NM_032173.4; 66-67	ACTTAATCTTAC-TAGGAAGGATCC	69.6 kb
148	21621-L30237	Exon 3	522-523	CTATGTAATAAC-AATGACGAAGAG	39.5 kb
256 +	21631-L30247	Intron 3	NR_046851.1; 598-597 *reverse*	ACTTGCATTCCA-TTGCTGTCTCCT	4.8 kb
310 +	21633-L30249	Intron 3	NR_046851.1; 65-64 *reverse*	TGTGCTCTTGGT-GTGTGACCTCAT	11.1 kb
214	21627-L30243	Exon 4	684-685	GAAAACCCAGAA-GCTATTGATCAG	0.8 kb
142	21620-L30236	Exon 5	774-775	AAGCTGATGAAC-ATCGTCAACAAG	1.4 kb
364	21636-L30252	Exon 6	869-870	TTTCCTGGCTTT-CTTCGTCGTGGT	2.1 kb
232	21628-L30244	Exon 7	1094-1095	TCTGGAGAAGTA-CATTGATGGAGA	1.5 kb
328	21634-L30250	Exon 8	1135-1136	CCTGTACTCACC-GGTTTCACAGGA	1.2 kb
184	21624-L30240	Exon 9	1593-1594	TCCCAGTATGAG-ACCATGTACCAG	0.6 kb
160	21622-L30238	Exon 9	2193-2194	CTGGAGATGAAC-TACAGCAGCAAC	3.4 kb
		stop codon	*3004-3006 (ex 10)*		
265	21632-L30248	Exon 10	3018-3017 *reverse*	CCAGGTAAGAGT-TCCTCCTGAGCT	6.9 kb
Flanking probes downstream of *ZNRF3*.					
195 « ¬	21625-L30241	*C22orf31*	22q12.1	GATTAAAGCACA-AGGACGATTCAG	60.9 kb
355 ¬	21635-L30251	*KREMEN1*	22q12.1	AACGTCCAACAA-ACTCACCATACA	94.1 kb
391 « ¬	21638-L30254	*EMID1*	22q12.2	CAGAACTGTGGT-GAGACCCACATA	172.9 kb
292 ¬	15529-L17384	*AP1B1*	22q12.2	CTTGGCACCAAA-ATGTCCGCGGCC	284.8 kb
165 ¬	21640-L22648	*NF2*	22q12.2	CACCGAGGAGGA-GGCAAAACTTCT	-

[a] See section Exon numbering on page 1 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.

« 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.
¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
+ Probe placed in *ZNRF3-AS1*, an antisense gene located in intron 3 of *ZNRF3*.

This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.

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

Table 3. Reference probes arranged according to chromosomal location.

Length (nt)	SALSA MLPA probe	Gene	Chromosomal band (hg18)	Partial sequence (24 nt adjacent to ligation site)	Location (hg18) in kb
409	14839-L30627	*UROD*	1p34	GGCTATGAGGTG-GTTGGGCTTGAC	01-045,253
377	20767-L31004	*F5*	1q24	CCTCAAACCATT-TAGAATGGGCAA	01-167,750
427	20872-L28890	*PSEN2*	1q42	GAGCTGACCCTC-AAATACGGAGCG	01-225,138
372	05953-L28763	*SPAST*	2p22	GCAAGTTGTGCT-AGTTCTTTTTGG	02-032,222
130	19551-L26105	*DYSF*	2p13	CCATTGCCAAGA-AGGTCAGTGTCC	02-071,750
154	13816-L15310	*EDAR*	2q13	CTCACATTCCTT-GGTGTTGGGGGG	02-108,906
208	21639-L15999	*SLC40A1*	2q32	GATTGACCAGTT-AACCAACATCTT	02-190,138
319	15385-L17792	*SCN5A*	3p22	GCAGATCAGAAA-CATGATGGTGAC	03-038,571
274	14827-L30626	*CPOX*	3q11	GTTTGGTGGTGG-ATGTGACCTCAC	03-099,790
172	21193-L29851	*PKHD1*	6p12	GGAAGATTGGAA-ACTTTTGATTTT	06-052,046
244	21641-L30683	*SIM1*	6q16	CCAAGTACTACA-GGTTCCTGGCGA	06-101,002
226	08064-L30628	*DNAI1*	9p13	AGGTGATTTCAG-AAACAGGAAACC	09-034,480
400	01237-L00568	*UPF2*	10p14	TGCCATTCCTTT-GCATCTCAAAAG	10-012,019
190	12409-L29450	*PCDH15*	10q21	AGTGAAGATTCT-TGTCTTACATCC	10-055,390
340	21099-L30929	*SLC6A5*	11p15	CCTCCATCTCAG-ACGAGTTTCCCA	11-020,615
433	05290-L04670	*ATL1*	14q22	GGTGTAGGGAAA-GCTTGATGATAC	14-050,169
284	06725-L06313	*HEXA*	15q24	AGTTGACATCTG-ACCTGACATTTG	15-070,425
136	11367-L12092	*SGCA*	17q21	AGAGACCTGGCT-ACCTCCGAGTGA	17-045,603

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

References

- Assié G et al. (2014). Integrated genomic characterization of adrenocortical carcinoma. *Nat Genet*. 46:607-12.
- Atanesyan L et al. (2017). Optimal fixation conditions and DNA extraction methods for MLPA analysis on FFPE tissue-derived DNA. *Am J Clin Pathol*. 147:60-8.
- Bond CE et al. (2016). RNF43 and ZNRF3 are commonly altered in serrated pathway colorectal tumorigenesis. *Oncotarget*. 7:70589-600.
- Fraser M et al. (2021) Somatic driver mutation prevalence in 1844 prostate cancers identifies ZNFR3 loss as a predictor of metastatic relapse. *Nat Commun*. 12:6248.
- Hao HX et al. (2012). ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature*. 485:195-200.
- Hömig-Hölzel C and Savola S. (2012). Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. *Diagn Mol Pathol*. 21:189-206.
- Juhlin CC et al. (2015). Whole-exome sequencing characterizes the landscape of somatic mutations and copy number alterations in adrenocortical carcinoma. *J Clin Endocrinol Metab*. 100:E493-502.
- Nord KH et al. (2013). Recurrent chromosome 22 deletions in osteoblastoma affect inhibitors of the Wnt/beta-catenin signaling pathway. *PLoS One*. 8:e80725.
- Robinson D et al. (2015). Integrative clinical genomics of advanced prostate cancer. *Cell*. 161:1215-28.
- 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.
- Zheng S et al. (2016). Comprehensive pan-genomic characterization of adrenocortical carcinoma. *Cancer Cell*. 29:723-36.

P476 product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-03 – 15 February 2022 (04P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Ligation sites of the probes targeting the *ZNRF3* gene updated according to new version of the NM_001206998.2 reference sequence. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). - Warning added to Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. Version A1-02 – 25 September 2018 (01P) - P476 specific note added on page 3. Version A1-01 – 09 May 2018 (01P) - Not applicable, new document.

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