Product Description
SALSA® MLPA® Probemix P438-D3 Celiac Disease

To be used with the MLPA General Protocol.

Version D3
As compared to version D2, three reference probes have been replaced and two probe lengths have been adjusted. In addition, the name of the probemix has been changed to SALSA MLPA Probemix P438 Celiac Disease. For complete product history see page 8.

Catalogue numbers:
- **P438-025R:** SALSA MLPA Probemix P438 Celiac Disease, 25 reactions.
- **P438-050R:** SALSA MLPA Probemix P438 Celiac Disease, 50 reactions.
- **P438-100R:** SALSA MLPA Probemix P438 Celiac Disease, 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.

Intended purpose
The SALSA MLPA Probemix P438 Celiac Disease is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative assay[2] for the detection of celiac disease (CD)-associated risk variants HLA-DQ2.5, HLA-DQ2.2 and HLA-DQ8 in genomic DNA isolated from human peripheral whole blood specimens. P438 Celiac Disease is intended to strengthen clinical diagnosis of CD and for molecular genetic testing of at-risk family members. P438 Celiac Disease can be used to exclude the possibility of CD in first-degree relatives and other at-risk groups. P438 is not intended to determine the copy number of the detected variant alleles.

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation or prenatal testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for in vitro diagnostic (IVD) use in the countries specified at the end of this product description. In all other countries, the product is for research use only (RUO).
[2] To be used in combination with a SALSA MLPA Reagent Kit, Coffalyser.Net analysis software, and SALSA Binning DNA SD089.

Clinical background
Celiac disease is a chronic, small intestinal enteropathy triggered by gluten proteins from wheat, barley and rye. It is characterized by an autoimmune response in genetically susceptible individuals, resulting in small intestine mucosal injury. CD affects roughly 1% of the population. Higher risk groups include individuals having an affected first-degree relative, and those with Down syndrome, Turner syndrome, selective immunoglobulin A (IgA) deficiency, type I diabetes mellitus or other autoimmune conditions.

CD is a multigenic disorder, in which the most dominant genetic risk factors are specific HLA-DQ variants encoded by the HLA-class II region on chromosome 6p. Presence of one of these risk variants is necessary but not sufficient to cause the disease, as other genes (many of which are involved in the immune system or in intestinal permeability) and environmental factors play a role as well (Barakauskas et al. 2014). Although the CD risk variants have a combined prevalence of up to 40% in the general population (20-30% for DQ2.5 and DQ2.2 combined; 10% for DQ8), CD is extremely rare in individuals lacking all of them.

The HLA-DQ2.5 variant is present in 90-95% of patients with CD and is encoded by the *HLA-DQA1*05* / *HLA-DQB1*02* alleles. The remaining patients are positive for either the HLA-DQ8 variant (encoded by *HLA-DQA1*03* / *HLA-DQB1*0302*) or the HLA-DQ2.2 variant (encoded by *HLA-DQA1*02* / *HLA-DQB1*02)* (Tack et al. 2010; Mubarak et al. 2013). All aforementioned alleles can be detected by this MLPA probemix (see Table 1). In addition, the HLA-DQ7.5 variant, encoded by *HLA-DQA1*05* / *HLA-DQB1*0301*, was suggested to be involved in CD (Martínez-Ojinaga et al. 2018), but this has not yet been established. The HLA-DQ7.5 variant can be tentatively identified with this MLPA probemix (see Interpretation of results section for more information).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1727/.

Gene structure
The *HLA-DQA1* and *HLA-DQB1* genes are located on chromosome 6p21.32. *HLA-DQA1* spans 6.2 kilobases (kb) and contains 5 exons (GenBank NG_032876.1), and *HLA-DQB1* spans 6.8 kb and contains 5 exons (GenBank NG_029922.1).

Transcript variants
For *HLA-DQA1*, one transcript variant has been described encoding the full length protein (NM_002122.5; 1574 nucleotides (nt); coding sequence 54-821; http://www.ncbi.nlm.nih.gov/gene/3117). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 4.

For *HLA-DQB1*, multiple variants have been described. Transcript variant 1 is the most predominant and encodes isoform 1 (NM_002123.5; 1605 nt.; coding sequence 51-836; http://www.ncbi.nlm.nih.gov/gene/3119). This sequence is a reference standard in the NCBI RefSeq project. The ATG translation start site is located in exon 1 and the stop codon is located in exon 5.

Exon numbering
The *HLA-DQA1* and *HLA-DQB1* exon numbering used in this P438-D3 Celiac Disease product description is the exon numbering from the NG_032876.1 and NG_029922.1 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 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 P438-D3 Celiac Disease contains 20 MLPA probes with amplification products between 130 and 344 nt. This includes 11 probes specific for the detection of *HLA-DQA1* and *HLA-DQB1* alleles for the subtypes mentioned in Table 3, which will only generate a signal when the allele is present. In addition, nine reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.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 reference probes over the experiment.

Required specimens
Extracted DNA from human 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
As individual samples cannot contain all three HLA-DQ risk variants, it is impossible to select suitable reference samples containing the target sequences of all probes. Therefore, data normalisation is done without dedicated reference samples. Instead, each sample should be normalised against all other samples in the same experiment (we recommend testing a minimum of eight samples in each MLPA experiment). This allows reliable detection of the presence of all HLA-DQ risk variants. However, it does not allow reliable determination of variant copy number. All samples tested should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible.

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample for each of the HLA-DQ risk variants 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.

SALSA Binning DNA SD089
The SD089 Binning DNA provided with this probemix can be used for binning of all probes including the 11 HLA-DQ allele-specific probes (see Table 3). SD089 Binning DNA is a mixture of female genomic DNA from a selected cell line and plasmid DNA that together contain all target sequences detected by this probemix. Inclusion of one reaction with 5 µl SD089 Binning DNA in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net software. Furthermore, Binning DNA should be included in the experiment whenever changes have been applied to the set-up of the capillary electrophoresis device (e.g. when capillaries have been renewed). Binning DNA should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of the allele-specific signals. It is strongly advised that all samples tested are extracted with the same method and derived from the same source of tissue. For further details, please consult the SD089 Binning DNA product description, available online: www.mrcholland.com.

Performance characteristics
Nearly 100% of CD patients express either the HLA-DQ2.5, HLA-DQ2.2 or HLA-DQ8 risk variants, or a combination of these variants, and all three of these can be identified by this MLPA probemix. The remaining

patients may carry the HLA-DQ7.5 variant, which can be tentatively identified by the P438 probemix (see Interpretation of results section for more information). The analytical sensitivity and specificity for the detection of the CD-associated HLA-DQ risk variants is very high and can be considered >99% (based on a 2013-2019 literature review).

Analytical performance can be compromised by: SNVs or other polymorphisms in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

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

SALSA MLPA Probemix P438 Celiac Disease is intended to detect absence or presence of the CD-associated risk variants DQ2.5, DQ2.2 and DQ8. The standard deviation of each individual reference probe over all the samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the samples should be between 0.80 and 1.20. Table 1 and 2 can be used for HLA-DQ variant annotation, describing single and combined variants within one sample and specific HLA-DQB1 variants, respectively.

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 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.

Table 1. Expected probe signals in different HLA-DQ variants

Length (nt)	SALSA MLPA probe	HLA allele	DQ2.5	DQ2.2	DQ8	DQ7.5
202	11292-L12018	DQA1*05	+	-	-	+
332	11293-L12019	DQA1*05	+	-	-	+
184	11296-L12022	DQB1*02	+	+	-	-
319	11295-L12021	DQB1*02	+	+	-	-
222	19115-L25062	DQA1*02	-	+	-	-
287	22800-SP0747-L25064	DQA1*02	-	+	-	-
178	11289-L12015	DQA1*03	-	-	+	-
144	S0371-SP0074-L12959	DQA1*03	-	-	+	-
229	20917-SP0075-L12960	DQB1*03	-	-	+	+
136	S0460-SP0135-L15177	DQB1*0302 *0305	-	-	+	-
256	23049-SP0849-L32517	DQB1*0302 *0303	-	-	+	-

The table indicates for each probe whether a signal is expected for the different HLA-DQ risk variants (+) or not (-). Presence of two risk variants leads to a combination of the indicated probe signals.

Table 2. Expected probe signals in specific HLA-DQB1*03 variants

Length (nt)	SALSA MLPA probe	HLA allele	B1*0301	B1*0302	B1*0303	B1*0305
229	20917-SP0075-L12960	DQB1*03	+	+	+	+
136	S0460-SP0135-L15177	DQB1*0302 *0305	-	+	-	+
256	23049-SP0849-L32517	DQB1*0302 *0303	-	+	+	-

- False positive results: Please note that abnormalities detected by a single probe 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.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.

Additional information on P438 data interpretation:

P438 data analysis without dedicated reference samples can lead to large confidence intervals in the ratio chart, statistically ambiguous results or extremely high final ratios for the target probes, interpretation of which is further explained below.

Note: if a final ratio is given for a certain target probe, this means that presence of the corresponding allele has been detected. The second probe for the same allele can be used to confirm this result. Background signals are shown as a percentage and do not indicate presence of the allele.

- Data analysis without dedicated reference samples in an experiment detecting alleles with a combined prevalence of up to 40% in the general population leads to high standard deviations and large confidence intervals for all target probes. This is inherent to the analysis method and does not indicate bad sample or probe quality. However, the standard deviation of all reference probes should be <0.10.
- A final ratio outside the arbitrary borders (0.7-1.3) but not significantly different from the sample population will be called ambiguous by Coffalyser.Net. Furthermore, if the majority of the samples in the sample population is negative for a certain HLA-DQ allele, the probe result in samples positive for this allele will be called ambiguous, due to a lack of reference signals for this probe. In both cases, this ambiguity does not prohibit further analysis of the P438 results.
- Background signals occur relatively frequently using the P438 probemix due to the small number of probes included. Occurrence can be decreased as described in the P438 specific notes below. However, if one sample in the experiment is positive for a certain target probe and one or more other samples have a background signal at the position of this probe, a very high final ratio is calculated for the probe with the positive signal. This situation is more likely to occur in small sample sets. Therefore we recommend testing a minimum of eight samples in each experiment. The high final ratio can still be interpreted as presence of the HLA-DQ allele targeted by the corresponding probe.
- We always recommend visual inspection of the size-called peak pattern to aid in interpretation of P438 results, especially when a relatively small number of samples is included in the experiment.

P438 specific notes:

- As all P438 target probes are designed to bind to the extremely polymorphic HLA-DQ region, almost every position of the target sequence is variable. For all target probes, and in particular for the probes at 136 nt and 256 nt detecting DQB1*0302 *0305 and DQB1*0302 *0303, respectively, there is a possibility that they additionally target (very rare) variants. We have not encountered this in the validation studies.
- In our quality tests, we have encountered a sample showing an increased signal for the 229 nt probe and normal signal for the 136 nt and 256 nt probes. Two possible explanations could be: one *HLA-DQB1*0301* and one *HLA-DQB1*0302* allele or one *HLA-DQB1*0303* and one *HLA-DQB1*0305* allele.
- HLA-DQ7.5 can be tentatively identified. A probe specific for *HLA-DQB1*0301* is not included, but the probemix does contain a probe detecting all *HLA-DQB1*03xx* alleles. If the probes detecting the DQB1 *0302, *0303 and *0305 alleles do not give a signal, but the HLA-DQB1*03xx probe does give a signal, the probability that the sample is positive for the *HLA-DQB1*0301* allele is high. The presence of this allele in combination with *HLA-DQA1*05* would detect the HLA-DQ7.5 variant.

- This probemix contains a relatively low number of probes, which may lead to off-scale peaks and/or high noise level. This is more likely to occur in samples that are negative for all or most of the HLA-DQ variants targeted by this probemix. Coffalyser.Net software warns for off-scale peaks and a high amount of noise peaks while other software does not. If one or more peaks are off-scale or if a high amount of noise peaks is observed, 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. For more information, please contact info@mrcholland.com.

Confirmation of results

HLA-DQ alleles detected by only a single probe always require confirmation by another method. The apparent presence of an HLA-DQ allele detected by a single probe can be due to e.g. a mutation/polymorphism that allows ligation on additional rare HLA-DQ variants. Sequence analysis can establish whether rare variants are present.

HLA-DQ alleles detected by more than one target probe should be confirmed by another independent technique such as sequence-specific oligonucleotide primed PCR (PCR-SSO), sequence-specific primer-based PCR (PCR-SSP) or sequencing.

Please report copy number changes detected by the reference probes, false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.

Table 3. SALSA MLPA Probemix P438-D3 Celiac Disease

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*HLA-DQA1* variant	*HLA-DQB1* variant
64-105	Control fragments – see table in probemix content section for more information			
130	Reference probe 16316-L18705	3q		
136 Ж	**HLA-DQB1 probe** S0460-SP0135-L15177			***0302 *0305**
144 Ж	**HLA-DQA1 probe** S0371-SP0074-L12959		***03**	
160 *	Reference probe 19762-L26545	9q		
172 *	Reference probe 09940-L29795	8q		
178	**HLA-DQA1 probe** 11289-L12015		***03**	
184	**HLA-DQB1 probe** 11296-L12022			***02**
193	Reference probe 05986-L05411	20p		
202	**HLA-DQA1 probe** 11292-L12018		***05**	
214	Reference probe 16426-L18879	18q		
222	**HLA-DQA1 probe** 19115-L25062		***02**	
229 Ж	**HLA-DQB1 probe** 20917-SP0075-L12960			***03**
241	Reference probe 05658-L05111	2p		
256 ¥ Ж	**HLA-DQB1 probe** 23049-SP0849-L32517			***0302 *0303**
274	Reference probe 08053-L07834	5p		
287 ¥ Ж	**HLA-DQA1 probe** 22800-SP0747-L25064		***02**	
304 *	Reference probe 17066-L26124	7q		
319	**HLA-DQB1 probe** 11295-L12021			***02**
332	**HLA-DQA1 probe** 11293-L12019		***05**	
344	Reference probe 06708-L06295	10p		

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

* New in version D3.
¥ Changed in version D3. Minor alteration, no change in sequence detected.
Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

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 4. HLA-DQ probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	Exon[a]	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
			***HLA-DQA1* gene**		
			NM_002122.5		
		start codon	*54-56 (Exon 1)*		
178	11289-L12015	Exon 2	191-192	GGTCCCTCTGGG-CAGTACAGCCAT	0.07 kb
222	19115-L25062	Exon 2	261-262	AGACTGTCTGGA-AGTTGCCTCTGT	0.05 kb
144 Ж	S0371-SP0074-L12959	Exon 2	313-314, 348-349	ATTTGCACTGAC-35 nt spanning oligo-TGATTAAACGCT	0.3 kb
202	11292-L12018	Exon 2	189 nt after exon 2	TAAATCCTTCTC-GGAGAGGTCTCA	0.4 kb
332	11293-L12019	Exon 3	610-611	TGCTGAGGAGAG-TTATGACTGCAA	0.4 kb
287 Ж	22800-SP0747-L25064	Exon 4	716-715, 683-682 reverse	AACCCCAGGGCA-33 nt spanning oligo-GCTGGAATCTCA	18.8 kb
		stop codon	*819-821 (Exon 4)*		
			***HLA-DQB1* gene**		
			NM_002123.5		
		stop codon	*834-836 (Exon 5)*		
184	11296-L12022	Exon 4	37 nt before exon 4	AACTATGGGGTA-TGGGGACAAACA	3.4 kb
229 Ж	20917-SP0075-L12960	Exon 2	367-368, 399-400	GACCCGGGCGGA-32 nt spanning oligo-TGGAGCTCCGCA	0.05 kb
136 Ж	S0460-SP0135-L15177	Exon 2	317-316, 287-286 reverse	CAGTACTCGGCG-30 nt spanning oligo-TACACCCCCACG	0.04 kb
319	11295-L12021	Exon 2	251-252	TATAACCGAGAA-GAGATCGTGCGC	0.08 kb
256 Ж	23049-SP0849-L32517	Exon 2	27 nt before exon 2, 172-173	GGGCCGGGGCCT-40 nt spanning oligo-CCAGTTTAAGGG	
		start codon	*51-53 (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.

Ж This probe consists of three parts and has two ligation sites. A low signal of this probe can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.

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

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- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
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Selected publications using SALSA MLPA Probemix P438 Celiac Disease

- Rouvroye MD et al. (2019). HLA-DQ Typing Kits in Diagnosis and Screening for Celiac Disease. *Genet Test Mol Biomarkers.* 23(6):418-422.
- Van Beek EM et al. (2013). A multiplex assay to rapidly exclude HLA-DQ2.5 and HLA-DQ8 expression in patients at risk for celiac disease. *Clin Chem Lab Med.* 51(6):1191-1198.
- Vijzelaar R et al. (2016). Rapid Detection of the Three Celiac Disease Risk Genotypes HLA-DQ2.2, HLADQ2.5, and HLA-DQ8 by Multiplex Ligation-Dependent Probe Amplification. *Genet Test Mol Biomarkers.* 20(3):158-161.

P438 product history	
Version	*Modification*
D3	Three reference probes have been replaced and two probe lengths have been adjusted. Name of the probemix has been changed from SALSA MLPA Probemix P438 HLA to SALSA MLPA Probemix P438 Celiac Disease.
D2	First unrestricted release.

Implemented changes in the product description

Version D3-01 – 12 May 2022 (04P)
- Product description rewritten and adapted to a new template.
- P438-D3 is now CE marked.
- Product description adapted to a new product version (version number changed, changes in Table 1, 2, 3 and 4).
- All salt warnings removed from Table 3 and 4, as they are not relevant anymore.
- Note added on how to handle off-scale peaks and high noise level.
- Additional information on P438 data interpretation added to section Interpretation of results.
- Name of the probemix has been changed to SALSA MLPA Probemix P438 Celiac Disease.

Version D2-03 – 20 March 2020 (02P)
- Product description adapted to a new template.
- Sections Reference samples, Positive control DNA samples, SALSA Binning DNA and Data analysis rewritten to clarify data interpretation.
- Added HLA-DQ7.5 variant to General information and Table 1.
- Updated NM_sequences for HLA-DQA1 and HLA-DQB1 in Table 4.
- Updated Ligation sites for HLA-DQB1 in Table 4.
- Added four new references.

Version D2-02 – 15 May 2018 (01P)
- Minor textual change to the table 3 title.

Version D2-01 – 16 March 2018 (01P)
- Product description restructured and adapted to a new template.

More information: www.mrcholland.com; www.mrcholland.eu	
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IVD	EUROPE* CE
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA), and the UK. The product is for RUO in all other European countries.