Instructions for Use

SALSA® MLPA® Probemix P438 Celiac Disease

See also the MLPA General Protocol, and the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Binning DNA SD089, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P438 Celiac Disease product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P438 Celiac Disease**
Version	D3
Catalogue numbers	P438-025R (25 reactions) P438-050R (50 reactions) P438-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Additional Test Components	**Catalogue Numbers**
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM
SALSA® Binning DNA SD089	SD089

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

Regulatory Status	
IVD	EUROPE CE
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State in which the user and/or the patient is located.

Changes in this Product Version

As compared to version D2, 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.

1. 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 IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types	
Test Sample	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test samples • At least eight independent samples required in each experiment for proper data normalisation • Have a normal copy number and ≤0.10 standard deviation for all reference probes
Reference Samples (Not Required*)	• Data normalisation is done without dedicated reference samples • Each test sample should be normalised against all other test samples in the same experiment
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination
Binning Sample (Initial Experiment)	• SALSA Binning DNA SD089, provided by MRC Holland • Required in initial experiment to determine suitable bin set • Should never be used as a reference sample
Positive Control Samples (Preferably)	• Provided by user • Recommended to include a positive sample for each of the HLA-DQ risk variants in each experiment

* Please note that it is impossible to select suitable reference samples containing the target sequences of all probes, as individual samples cannot contain all three HLA-DQ risk variants.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Normal and Affected Populations
The typical final ratio (FR) values stated in the copy number table were determined in a validation study with samples containing normal copy numbers. The standard deviation of each individual reference probe over all the samples was ≤0.10.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

The table illustrates the relationship between final probe ratio and corresponding copy number. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

Possible Results of *HLA-DQA1* and *HLA-DQB1* allele-specific Probes

Signal Strength	Allele Status*
≥10% median peak height reference probes	Allele detected
<10% median peak height reference probes	Allele **not** detected

* 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.

For a detailed interpretation guide, see the Appendix.

6. 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 the Appendix 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 2013-2024 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.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Allelic variant	Length (nt)	Probe number	Warning
6p21.32	*HLA-DQA1*	Exon 2	0.07 kb	DQA1*03	178	11289-L12015	
6p21.32	*HLA-DQA1*	Exon 2	0.05 kb	DQA1*02	222	19115-L25062	
6p21.32	*HLA-DQA1*	Exon 2	0.3 kb	DQA1*03	144	S0371-SP0074-L12959	Ж
6p21.32	*HLA-DQA1*	Intron 2 (Exon 2)	0.4 kb	DQA1*05	202	11292-L12018	Ø
6p21.32	*HLA-DQA1*	Exon 3	0.4 kb	DQA1*05	332	11293-L12019	
6p21.32	*HLA-DQA1*	Exon 4	18.8 kb	DQA1*02	287	22800-SP0747-L25064	¥ Ж
6p21.32	*HLA-DQB1*	Exon 4	3.4 kb	DQB1*02	184	11296-L12022	
6p21.32	*HLA-DQB1*	Exon 2	0.05 kb	DQB1*03	229	20917-SP0075-L12960	Ж
6p21.32	*HLA-DQB1*	Exon 2	0.04 kb	DQB1*0302 *0305	136	S0460-SP0135-L15177	Ж
6p21.32	*HLA-DQB1*	Exon 2	0.08 kb	DQB1*02	319	11295-L12021	
6p21.32	*HLA-DQB1*	Exon 2		DQB1*0302 *0303	256	23049-SP0849-L32517	¥ Ж
2p	Reference				241	05658-L05111	
3q	Reference				130	16316-L18705	
5p	Reference				274	08053-L07834	
7q	Reference				304	17066-L26124	*
8q	Reference				172	09940-L29795	*
9q	Reference				160	19762-L26545	*
10p	Reference				344	06708-L06295	
18q	Reference				214	16426-L18879	
20p	Reference				193	05986-L05411	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland.
The *HLA-DQA1* and *HLA-DQB1* exon numbers are derived from the MANE project and based on the MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotation of one probe with a target at the edge of or slightly outside the coding region, is altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18

7. Precautions and Warnings

Probe changes

*	New probe.
¥	Probe changed in this product version. Minor alteration, no change in sequence detected.

Probe warnings

Ж	These probes consist of three parts and have two ligation sites. A low signal of these probes can be due to depurination of the sample DNA, e.g. due to insufficient buffering capacity or a prolonged denaturation time.
Ø	This probe targets a sequence outside of the known coding region.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Small changes (e.g. SNVs, small indels) in the sequence targeted by a reference probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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.
3. 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. Please report false positive results due to SNVs and unusual results to MRC Holland: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The allele-specific probes can only detect the presence of the allele and should not be used to determine zygosity.
2. The P438 probemix cannot be used to determine whether the detected alleles are present *in cis* or *in trans*.
3. 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. This has not been encountered in the validation studies.
4. HLA-DQ7.5 can be tentatively identified in a research setting. A probe specific for HLA-DQB1*0301 is not included, but the probemix does contain a probe detecting all HLA-DQB1*03 alleles. If the probes detecting the DQB1 *0302, *0303 and *0305 alleles do not give a signal, but the general HLA-DQB1*03 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.
5. 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.

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description
Version D3-02 – 19 February 2025 (03S) - Product description rewritten and adapted to a new template. - Reference to SALSA Binning DNA SD089 removed from the intended purpose footnote. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Warning for a probe targeting a sequence outside of the known coding region included for probe 11292-L12018. - Added a limitation that allele-specific probes cannot be used to determine zygosity. - Added a limitation stating that the probemix cannot determine whether the detected alleles are present *in cis* or *in trans*.

9. Appendix

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 tables below can be used for HLA-DQ variant annotation, describing single and combined variants within one sample and specific HLA-DQB1 variants, respectively.

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.

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	-	+	+	-

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.

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.

- 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.

- 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.