Instructions for Use

SALSA® MLPA® Probemix P236 CFH Region

See also the MLPA General Protocol, the product descriptions of the SALSA® MLPA® Reagent Kit, SALSA® Reference Selection DNA SD072 and the Coffalyser.Net Reference Manual.
Visit the SALSA® MLPA® Probemix P236 CFH Region product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P236 CFH Region**
Version	B1
Catalogue numbers	P236-025R (25 reactions) P236-050R (50 reactions) P236-100R (100 reactions)
Basic UDI-DI	872021148P23663
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® Reference Selection DNA SD072	SD072

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 2797
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 or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version A3, the name of the product has been changed from ARMD mix-1 to CFH Region. Four new probes for *CFHR4* and five new probes for *CFH* have been included, and several target probes have been replaced. One additional probe for *CFHR1* and two additional probes for *CFHR5* have been included. Most reference probes have been replaced and the flanking probes have been removed. The probes detecting polymorphic sequences have been removed.

1. Intended Purpose

The SALSA MLPA Probemix P236 CFH Region is an in vitro diagnostic (IVD)[1] or research-use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* genes in genomic DNA isolated from human peripheral whole blood specimens. P236 CFH Region is intended to confirm a potential cause for and clinical diagnosis of genetic atypical hemolytic uremic syndrome (aHUS), to confirm a potential cause for C3 glomerulopathy (C3G), and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P236 CFH Region should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *CFH* gene are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis. Not all exons of the aforementioned genes are covered.

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, 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	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • Have two copies of all target sequences. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated individuals from families without a history of aHUS and C3G.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Reference Selection DNA	• SALSA Reference Selection DNA SD072, available from MRC Holland • Use SD072 to identify suitable reference samples in initial experiments • **SD072 should never be used as a reference sample.**	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• This probemix contains target probes that target sequences with natural variation. The validation experiments of this probemix should result in a standard deviation ≤0.10 for all reference probes.	

*When testing >21 samples, include one extra reference for each 7 test samples.

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 tables were determined in a validation study with samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

Expected Results of Reference Probes

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

Typical Results of Probes Targeting Two Copies (*CFH, CFHR1, CFHR2, CFHR3, CFHR4* and *CFHR5 genes*)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final 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.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table above can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P236 CFH Region in 46 samples from healthy individuals with normal copy number or copy number status of 0 or 1 (in about 15% of healthy individuals, *CFHR3* is deleted due to homologous recombination–most often together with *CFHR1* (Pouw et al. 2016)) and 45 samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Limit of detection	A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. The use of insufficient or too much sample DNA can affect performance. These lower and higher limits of detection were verified using SALSA MLPA Probemix P236 CFH Region on two samples with known CNVs and on one sample with a normal copy number of all targets, and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA and hemoglobin) can affect the MLPA reaction. A study using SALSA MLPA Probemix P236 CFH Region was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 317/342 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 339/342 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 321/342 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 342/342 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 137/342 measurements

* Results are summarised for 38 target probes across all three samples tested.

	Hemoglobin had the largest effect on copy number determination: FRs with an incorrect range were obtained in both a *CFH* region duplication sample and the wildtype sample. In the sample harbouring a *CFH* region deletion, ambiguous FRs were obtained. For such values that are not within an annotated cut-off range, no false positives or negatives would ensue, but results may be delayed due to potential decisions to repeat the assay. DNA extraction methods from blood remove hemoglobin and during testing of 23 samples extracted from blood the expected FRs were found in all but one measurement. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found. Importantly, warnings or errors were obtained in all affected samples using Coffalyser.Net software. To minimise variability across samples, 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.
Cross-reactivity	Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, 45 previously genotyped samples were tested using SALSA MLPA Probemix P236 CFH Region and found to have the expected results in 98s% of cases. Assay precision was tested by repeatedly testing samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 1666/1672 data points, leading to a precision of 99%.
Clinical validity*	30-32% of genetic aHUS cases are caused by structural variants such as deletions, duplications and hybrid genes in the *CFH* region *(CFH, CFHR3, CFHR1, CFHR4, CFHR2* and *CFHR5* genes*)* (GeneReviews). Less than 1% of C3G is caused by structural variants such as deletions, duplications and hybrid genes in the *CFH* region (*CFH, CFHR3, CFHR1, CFHR4, CFHR2* and *CFHR5* genes) (GeneReviews) *Based on a 2017-2024 literature review

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
1q31.3	*CFH*	Exon 1	20.9 kb	202	07820-L07574	
1q31.3	*CFH*	Exon 2	0.9 kb	142	07821-L07575	
1q31.3	*CFH*	Exon 3	2.1 kb	179	07822-L07576	
1q31.3	*CFH*	Exon 4	3.7 kb	419	07823-L16758	
1q31.3	*CFH*	Exon 6	23.4 kb	337	07824-L07578	
1q31.3	*CFH*	Intron 9	13.7 kb	292	22079-L31050	* # Ø
1q31.3	*CFH*	Intron 11	8.4 kb	373	07827-L07582	Ø
1q31.3	*CFH*	Exon 12	1.6 kb	310	07828-L07583	
1q31.3	*CFH*	Exon 14	1.6 kb	214	22074-L31045	*
1q31.3	*CFH*	Exon 15	9.1 kb	172	22071-L31042	*
1q31.3	*CFH*	Exon 17	3.2 kb	382	07830-L07586	
1q31.3	*CFH*	Exon 18	1.3 kb	208	22073-L31044	*
1q31.3	*CFH*	Exon 19	3.7 kb	472	22559-L31056	*
1q31.3	*CFH*	Exon 21	2.1 kb	436	22082-L31053	* + #
1q31.3	*CFH*	Downstream (Exon 22)	8.5 kb	324	22044-L31698	¥ # Ø
1q31.3	*CFH*	Downstream	12.5 kb	139	22043-L08618	¥ # Ø
1q31.3	*CFHR3*	Upstream	4.7 kb	135	22996-L32432	¥ # Ø
1q31.3	*CFHR3*	Upstream	1.5 kb	238	22997-L32433	¥ Ø
1q31.3	*CFHR3*	Exon 1	4.1 kb	164	07832-L07588	#
1q31.3	*CFHR3*	Exon 2	1.0 kb	274	07833-L07589	+ #
1q31.3	*CFHR3*	Exon 3	8.6 kb	392	07834-L07590	+
1q31.3	*CFHR3*	Intron 4 (Exon 4)	0.7 kb	154	22069-L31040	* # Ø
1q31.3	*CFHR3*	Intron 4	4.1 kb	364	07835-L07591	# Ø
1q31.3	*CFHR3*	Exon 6	27.2 kb	168	08218-L09921	#
1q31.3	*CFHR1*	Intron 1	5.1 kb	283	22112-L31100	* # Ø
1q31.3	*CFHR1*	Exon 2	1.7 kb	494	22087-L31058	* #
1q31.3	*CFHR1*	Intron 3	0.5 kb	346	07839-L07595	# Ø
1q31.3	*CFHR1*	Intron 3 (Exon 4)	2.9 kb	196	22072-L31043	* # Ø
1q31.3	*CFHR1*	Exon 5	1.3 kb	244	22076-L31047	* +
1q31.3	*CFHR1*	Exon 6	55.9 kb	454	22995-L32431	* ∫ #
1q31.3	*CFHR4*	Upstream (Exon 1)	19.6 kb	445	22084-L31055	* Ø
1q31.3	*CFHR4*	Exon 5	2.9 kb	317	22994-L32539	* # Δ
1q31.3	*CFHR4*	Exon 6	8.1 kb	400	22558-L31052	*
1q31.3	*CFHR4*	Exon 10	26.2 kb	148	22111-L31098	*
1q31.3	*CFHR2*	Intron 1	5.1 kb	406	22113-L31101	* # Ø
1q31.3	*CFHR2*	Exon 2	1.6 kb	265	07842-L07598	+ #
1q31.3	*CFHR2*	Intron 3 (Exon 3)	6.7 kb	226	21368-L31327	¥ Ø
1q31.3	*CFHR2*	Exon 4	19.7 kb	184	07844-L07600	#
1q31.3	*CFHR5*	Exon 1	5.2 kb	330	07845-L30998	¥ #
1q31.3	*CFHR5*	Exon 2	1.1 kb	427	07846-L16757	#
1q31.3	*CFHR5*	Exon 3	18.6 kb	232	07847-L07603	#
1q31.3	*CFHR5*	Exon 8	6.0 kb	253	22077-L31048	*
1q31.3	*CFHR5*	Exon 10		481	22086-L31057	*
2p	Reference			220	08879-L08935	*
2q	Reference			414	12787-L20671	*
3p	Reference			500	09682-L22509	*
5q	Reference			130	00797-L00463	
9q	Reference			301	02767-L02196	
12q	Reference			157	02731-L01824	*
15q	Reference			190	03915-L03370	*
17q	Reference			258	16472-L26940	*
20p	Reference			355	05991-L05416	*
22q	Reference			463	12460-L13461	*

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 *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotations of several probes with targets at the edge of or slightly outside the coding region, were altered. The exon numbering from the previous version of this Product Description is disclosed between brackets when a discrepancy is present.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe changes

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

Probe warnings

Ø	These probes target sequences outside of the known coding region. Copy number alterations of one of these probes are of unknown clinical significance.
#	The specificity of these probes rely on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
+	The ligation site of these probes is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
∫	This probe can give a background signal in case of homozygous deletion of *CFHR1*. In the case of a heterozygous deletion of *CFHR1*, a signal slightly higher than expected can be obtained.
Δ	This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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. Sample or technical artefacts may appear as a (mosaic) copy number change of the whole/partial gene. Whole/partial gene deletions or duplications should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. Copy number alterations of reference probes are unlikely to be related to the condition tested for.
5. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. The *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4* and *CFHR5* genes have a high sequence homology, which makes the *CFH* region on 1q31.3 prone to gene conversions and recombination events. In rare cases, an apparent duplication of a single probe might be due to a sequence change in one of the homologous genes. In the Table delineating the content, the affected probes have an associated warning (#).

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Pouw RB et al. (2016). Complement factor H-related protein 3 serum levels are low compared to factor H and mainly determined by gene copy number variation in CFHR3. PLoS One. 11:e0152164.

Implemented changes in the product description
Version B1-03 – 18 June 2025 (03S) - The product description has been updated to a new template. - Intended purpose updated by removal of systemic lupus erythematosus (SLE). Confirm a clinical diagnosis limited to genetic aHUS. It has been specified that MLPA is a manual assay. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - SNVs rs191734103, rs145280059, rs16840465 and rs35292876 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Warning for sensitivity to certain experimental conditions added for probe 22994-L32539. - Performance characteristics updated based on data from analytical performance experiments. - Warnings for a ligation site >20 nt from the nearest exon were added for probes 22082-L31053, 07833-L07589, 07834-L07590, 22076-L31047 and 07842-L07598. - Warnings for probes targeting sequences outside of the known coding region were added for 22079-L31050, 07827-L07582, 22044-L31698, 22043-L08618, 22996-L32432, 22997-L32433, 22069-L31040, 07835-L07591, 22112-L31100, 07839-L07595, 22072-L31043, 22084-L31055, 22113-L31101 and 21368-L31327. - Probemix is now IVDR-certified.