Product Description SALSA® MLPA® Probemix P236-A3 ARMD mix-1

To be used with the MLPA General Protocol.

Version A3. As compared to version A2, one probe has a change in length. No change in sequence detected. Two control fragments at 88 and 96 nt have been replaced and two new control fragments at 100 and 105 nt have been included. For complete product history see page 9.

This SALSA® MLPA® probemix is for basic research and intended for experienced MLPA users only! This probemix enables you to quantify genes or chromosomal regions in which the occurrence of copy number changes is not yet well-established and the relationship between genotype and phenotype is not yet clear. Since it will not provide you with clear cut answers, interpretation of results can be complicated. MRC-Holland recommends thoroughly screening any available literature. Suggestions from specialists for improvement of this product or product description are highly appreciated.

Catalogue numbers:
- **P236-025R:** SALSA MLPA Probemix P236 ARMD mix-1, 25 reactions.
- **P236-050R:** SALSA MLPA Probemix P236 ARMD mix-1, 50 reactions.
- **P236-100R:** SALSA MLPA Probemix P236 ARMD mix-1, 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.mlpa.com).

Precautions and warnings: For professional use only. Always consult the most recent product description AND the MLPA General Protocol before use: www.mlpa.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.
This product requires the identification of suitable reference samples for proper data analysis. Suitable reference samples have 2 copies of all target sequences, including those in *CFHR1* and *CFHR3*.

General information: The SALSA MLPA Probemix P236 ARMD is a **research use only (RUO)** assay for the detection of deletions or duplications in the *CFH*, *CFHR3*, *CFHR1* and *CFHR2* genes, which are associated with age-related macular degeneration (ARMD), atypical hemolytic uremic syndrome (aHUS), polygenic autoimmune disease systemic lupus erythematosus (SLE) and C3 glomerulopathy. In addition, 6 probes for polymorphic sequences (SNPs) are present in the P236 probemix.

Age-related macular degeneration (ARMD) is a major cause of blindness in the elderly. ARMD is characterised by progressive destruction of the retina's central region (macula), causing central field visual loss. Contributions of environmental factors and genetic susceptibility have been identified. Up to 50% of the genetically attributable risk of age-related macular degeneration appears to be linked to the 1q31.3 chromosomal region, in particular to a copy number variation in the CFH region (*CFH*, *CFHR3*, *CFHR1*, *CFHR2* genes). A second major ARMD susceptibility allele was identified in the *ARMS2* gene (LOC387715) on 10q26 (SNP rs10490924). A third locus has been identified in the *C2*/*CFB* region (SNPs rs9332739 and rs641153). The *C2* and *CFB* genes are located on chromosome 6p21, encoding complement component 2 and complement factor B.

The *CFH*, *CFHR3*, *CFHR1* and *CFHR2* genes are arranged in tandem on chromosome 1q31.3, approximately 197 Mb from p-telomere, where they span 355 kb at the proximal end of a cluster of genes involved in regulation of the complement activation. A complement cascade is implicated in formation of drusen. Drusen are deposits that are formed between Bruch's membrane and the retinal pigment epithelium in eyes, which show an early sign of ARMD. Deletion of the *CFHR1* and *CFHR3* genes is common and has been linked to a lower risk on ARMD. This deletion was found in nearly 20% of the chromosomes of control individuals and only 7.8% of the chromosomes of individuals with ARMD (Hughes et al. 2006).

This P236-A3 ARMD probemix has also been used to measure copy number changes and to detect mutations in patients with aHUS (Moore et al. 2010). aHUS can be caused by mutations in several genes including *complement factor H* (*CFH*), *membrane cofactor protein CD46* (*MCP*) and *complement factor I* (*CFI*). The latter two genes are covered by probes in the P296 aHUS probemix. Probes for many *CFH* exons are present in this P236 probemix.

Copy number changes in the CFH region area are also associated with increased risks for SLE (Zhao et al. 2011) and the complement-mediated renal disease C3 glomerulopathy.

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 exon numbering used in this P236-A3 ARMD product description is the exon numbering from:
CFHR1: RefSeq transcript NM_002113.2, which is identical to the LRG_149 sequence.
CFHR2: RefSeq transcript NM_005666.4, which is identical to the LRG_1216 sequence.
CFHR3: RefSeq transcript NM_021023.5, which is identical to the LRG_175 sequence.
CFHR5: RefSeq transcript NM_030787.3, which is identical to the LRG_227 sequence.
CFH: The *CFH* exon numbering has changed. From description version A3-16 onwards, we have adopted the NCBI exon numbering that is present in the NM_000186.3 sequence for this gene, which is identical to the LRG_47 sequence. The exon numbering used in previous versions of this product description can be found between brackets in Table 2a.
C2: RefSeq transcript NM_000063.4, which is identical to the LRG_26 sequence.
ARMS2: RefSeq transcript NM_001099667.3.

The exon numbering and NM sequences have been retrieved in 10/2019. As changes to the NCBI database can occur after release of this product description, exon numbering may not be up-to-date.

Probemix content: The SALSA MLPA Probemix P236-A3 ARMD contains 49 MLPA probes with amplification products between 113 and 454 nucleotides (nt). This includes 13 probes for the *CFH* gene, 8 probes for *CFHR3*, 5 probes for *CFHR1* and 4 probes for *CFHR2*, as well as 5 probes in the flanking genes *KCNT2* and *CFHR5*. Furthermore, this probemix also contains 6 probes for polymorphic sequences (SNPs). For the relevance of these SNP specific probes for ARMD, we refer to the articles of Li et al. (2006) and Maller et al. (2006).
In addition, 8 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.mlpa.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.mlpa.com.

Length (nt)	Name
64-70-76-82	Q-fragments (only visible with <100 ng sample DNA)
88-96	D-fragments (low signal of 88 nt and 96 nt fragment 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.mlpa.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 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 healthy individuals. This product requires the identification of suitable reference samples for proper data analysis. Suitable reference samples have 2 copies of all target sequences, including those in *CFHR1* and *CFHR3*. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

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/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA00214 from the Coriell Institute has been tested with this P236-A3 probemix at MRC-Holland and can be used as a positive control sample to detect a deletion of the chromosomal region 1q31.3. 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.mlpa.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: Analysis of the results obtained with this probemix is complicated due to the abundance of SNPs and deletions in the *CFH-CFHR* region. Only a link between the frequency of certain chromosomal rearrangements or SNPs and the frequency of certain diseases has been reported. **MRC-Holland has no information on the significance of results obtained for the phenotype of the individual tested**.

We tested DNA samples from 41 healthy individuals and observed a heterozygous deletion of all *CFHR3* and *CFHR1* probes in 11 samples and a homozygous deletion of all *CFHR3* and *CFHR1* probes in four samples. In one sample we noticed a deletion of only the four *CFHR1* probes.

Signals for the 148 nt *CFHR3* exon 6 probe and the 282 nt *CFHR1* intron 1 probes appear to be influenced by other polymorphisms. These probes do not correlate with each other and do not always correlate with the copy number indicated by the other *CFFR1* and *CFHR3* probes. We recommend to disregard the results of these two probes. In the Coffalyser sheet, these probes have been placed at the bottom.

In addition to the probes covering the chromosomal 1q31.3 region downstream of the *CFH* gene, 6 probes for polymorphic sequences (SNPs) are present in the P236 probemix. Indicated below are our results on 50 different DNA samples from healthy individuals: (2 copies: 1 copy: 0 copy).

238 nt: *CFH* allele C of rs1061170 (minor allele of the Y402H polymorphism)	5 : 27 : 18
218 nt: *CFH* rs1410996 allele A (major allele)	7 : 25 : 18
157 nt: *ARMS2* (LOC387715) rs10490924 allele T (minor allele)	1 : 9 : 40
154 nt: *ARMS2* (LOC387715) rs10490924 allele G (major allele)	40 : 9 : 1

208 nt: *C2*/*CFB* loci rs9332739 polymorphism (minor allele) 0 : 4 : 46
211 nt: *C2*/*CFB* loci rs9332739 polymorphism (major allele) 46 : 4 : 0

The standard deviation of each individual reference probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) 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 DQ of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- 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. 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.
- 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.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. 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: lower injection voltage / injection time settings, 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 *CFH* are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P236 ARMD.
- 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. SNPs, point mutations, small indels) in the target sequence detected by a probe can cause false positive results. Mutations/SNPs (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.

Mutation database: There is a LOVD database including mutations for every gene (*CFH*, *CFHR1-5*): https://databases.lovd.nl/shared/genes/.
We strongly encourage users to deposit positive results in one of these databases. 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 SNPs and unusual results (e.g., a duplication of *CFH* exons 2 and 4 but not exon 3) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P236-A3 ARMD mix-1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]					
		reference	CFH	CFHR3	CFHR2	CFHR1	other
64-105	Control fragments – see table in probemix content section for more information						
113 #	**CFHR3 probe** S0259-L20945			Upstream			
117 #	**CFH probe** S0258-L08618		Downstream				
122 #	**CFH probe** S0257-L08617		Exon 22				
125	**CFHR3 probe** S0260-L16755			Upstream			
130	Reference probe 00797-L00463	5q31					
136	Reference probe 03571-L03264	7q31					
142	**CFH probe** 07821-L07575		Exon 2				
148 #	**CFHR3 probe** 07836-L07592			Exon 6			
154	**ARMS2 probe** 07848-L07604						rs10490924 major allele
157	**ARMS2 probe** 07848-L16762						rs10490924 minor allele
164 #	**CFHR3 probe** 07832-L07588			Exon 1			
168 #	**CFHR3 probe** 08218-L09921			Exon 6			
173	Reference probe 03578-L02939	7q31					
179	**CFH probe** 07822-L07576		Exon 3				
184 #	**CFHR2 probe** 07844-L07600				Exon 4		
191	**CFHR1 probe** 08216-L09922					Exon 6	
196	Reference probe 01218-L06270	22q11					
202	**CFH probe** 07820-L07574		Exon 1				
208	**C2 probe** 07849-L07606						rs9332739 minor allele
211	**C2 probe** 07849-L16761						rs9332739 major allele
218	**CFH probe** 07829-L16756		rs1410996 major allele				
226	**CFHR2 probe** 07843-L07599				Exon 3		
232 #	**CFHR5 probe** 07847-L07603						Exon 3
238	**CFH probe** 07851-L07610		rs1061170 minor allele				
247 «	Reference probe 01164-L00720	11q13					
256	**KCNT2 probe** 07819-L07573						Exon 3
265 #	**CFHR2 probe** 07842-L07598				Exon 2		
274	**CFHR3 probe** 07833-L07589			Exon 2			
282 #	**CFHR1 probe** 07837-L07593					Intron 1	
292	**CFH probe** 07826-L07581		Intron 9				
301	Reference probe 02767-L02196	9q22					
310	**CFH probe** 07828-L07583		Exon 12				
318	Reference probe 03263-L02700	3q29					
328	**CFHR5 probe** 07845-L07601						Exon 1
337	**CFH probe** 07824-L07578		Exon 6				
346 #	**CFHR1 probe** 07839-L07595					Exon 3	
355 ¬	**TNNT2 probe** 07777-L07533						Exon 17
364 #	**CFHR3 probe** 07835-L07591			Intron 4			
373	**CFH probe** 07827-L07582		Intron 11				
382	**CFH probe** 07830-L07586		Exon 17				
391	**CFHR3 probe** 07834-L07590			Exon 3			
399	**KCNT2 probe** 07818-L07572						Exon 1
406 #	**CFHR2 probe** 07841-L16760				Intro 1		
413	**CFHR1 probe** 07840-L16759					Exon 5	
419	**CFH probe** 07823-L16758		Exon 4				
427	**CFHR5 probe** 07846-L16757						Exon 2
433 ¬	**CRB1 probe** 06963-L06543						Exon 6
445	Reference probe 01050-L01836	8q24					
454 #	**CFHR1 probe** 08217-L07926					Exon 6	

a) See above section on exon numbering for more information.

¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
« 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.
This probe's specificity relies on a single nucleotide difference compared to a related gene. 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.

Notes:

- The 154-157, 208-211, 218 and 238 nt probes are specific for SNPs mentioned in several articles on ARMD, e.g. Li M. et al. 2006 *(Nat Genet. 38:1049-54)* and Maller J. et al. 2006 (*Nat Genet. 38:1055-9)*.
- Exon numbering used here may differ from literature! Please notify us of any mistakes. The identity of the genes detected by the reference probes is available on request: info@mlpa.com.

Table 2. P236-A3 probes arranged according to chromosomal location

Table 2a. Chromosome 1q31.3

Length (nt)	SALSA MLPA probe	Gene[a] exon	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		KCNT2	**NM_198503.5**		
		stop codon	*3537-3539 (Exon 28)*		
256	07819-L07573	Exon 3	401-400 *reverse*	CCTTACCATTCA-TTTCCTTGTGAA	118.4 kb
399	07818-L07572	Exon 1	188-189	AGGTTTCGAGAT-TTGCTGCTAGGG	43.9 kb
		start codon	*132-134 (Exon 1)*		
		CFH	**NM_000186.4**		
		start codon	*76-78 (Exon 1)*		
202	07820-L07574	Exon 1	120-119 *reverse*	TCTGCTACACAA-ATAGCCCATAAC	20.9 kb
142	07821-L07575	Exon 2	194-195	CTGGTCTGACCA-AACATATCCAGA	0.9 kb
179	07822-L07576	Exon 3	357-358	ACTCCTTTTGGT-ACTTTTACCCTT	2.1 kb
419	07823-L16758	Exon 4	458-459	TAATTACCGTGA-ATGTGACACAGA	3.7 kb
337	07824-L07578	Exon 6	818-819	TGAAAGAGGAGA-TGCTGTATGCAC	10.4 kb
238 §	07851-L07610	Exon 9 rs1061170	1279-1280	ATAATCAAAATC-ATGGAAGAAAGT	18.2 kb
292	07826-L07581	Intron 9 (10)	5429 nt before exon 10 reverse	GATAGGTAGTCA-TATTTGGAACAT	8.6 kb
373	07827-L07582	Intron 11 (12)	1079 nt after exon 11	CTTGGACACATT-ATGATTGAGTCG	8.4 kb
310	07828-L07583	Exon 12 (13)	1893-1894	ATAGTTGGACCT-AATTCCGTTCAG	2.6 kb
218 ∞	07829-L16756	Intron 14 (15) rs1410996	542 nt before exon 15	ATAGCTGAGTGA-CATGAGGTAGTC	9.7 kb
382	07830-L07586	Exon 17 (18)	2719-2720	ACGGAACCATTA-ATTCATCCAGGT	10.3 kb
122 #	S0257-L08617	Exon 22 (23)	282 nt after exon 22	TATCAATACATA-AATGCACCAAAA	8.5 kb
117 #	S0258-L08618	Downstream	8771 nt after exon 22	TGCACTTATACA-TGCAATCCGTTG	12.5 kb
		stop codon	*3769-3771 (Exon 22)*		
		CFHR3	**NM_021023.5**		
		start codon	*88-90 (Exon 1)*		
113 #	S0259-L20945	Upstream	6014 nt before exon 1	TTAGTCCGAGGT-AGAAAGGGACAT	4.7 kb
125	S0260-L16755	Upstream	1354 nt before exon 1	GGGTGGTAATCT-TGGCTCTCAGTG	1.5 kb
164 #	07832-L07588	Exon 1	8 nt after exon 1	CAAGGTAAGTTA-AAAGAGATCTAA	4.1 kb
274	07833-L07589	Exon 2	100 nt before exon 2 reverse	AACATTTTCTTG-TGGAATTACAGC	1.0 kb
391	07834-L07590	Exon 3	61 nt after exon 3	CACGGACGACAG-TCTCAGACTTGT	9.3 kb
364 #	07835-L07591	Intron 4	680 nt before exon 5	GGGGGTTATATG-AATTCCTACATT	3.8 kb
148 * #	07836-L07592	Exon 6	190 nt before exon 6 reverse	ACTTCCCCAACA-TCACAGCAGAGA	0.3 kb
168 #	08218-L09921	Exon 6	1045-1044 reverse	TATCCCTTCCCG-ACACACTGCTTG	26.9 kb
		stop codon	*1078-1080 (Exon 6)*		

Length (nt)	SALSA MLPA probe	Gene[a] exon	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		CFHR1	**NM_002113.2**		
		start codon	*115-117 (Exon 1)*		
282 * #	07837-L07593	Intron 1	449 nt after exon 1 reverse	AAGGATAATTCA-ATTGAAATGGGA	7.1 kb
346 #	07839-L07595	Intron 3	396 nt after exon 3	AGAGAGTTTCAG-GTCCATGTGTAG	3.4 kb
413 #	07840-L16759	Exon 5	75 nt after exon 5 reverse	ATAATCTGTGAT-TATTTTGTTACC	1.1 kb
191 #	08216-L09922	Exon 6	983-982 reverse	CACCTGTTCTCA-AATAAAGCTTCT	0.3 kb
454 #	08217-L07926	Exon 6	1284-1283 reverse	AGTTTTCCAAGT-TTTAATATGGTG	112.5 kb
		stop codon	*1105-1107 (Exon 6)*		
		CFHR2	**NM_005666.4**		
		start codon	*144-146 (Exon 1)*		
406 #	07841-L16760	Intron 1	730 nt after exon 1	TATGTCTGTACT-TGGAGTTTCGAT	5.1 kb
265 #	07842-L07598	Exon 2	74 nt after exon 2	CAAGATCATAAA-CACTTGATAATC	1.6 kb
226	07843-L07599	Exon 3	268 nt after exon 3	GTAATACCTGTG-TGTGGTTTATAG	6.7 kb
184 #	07844-L07600	Exon 4	655-656	ATATGCTCCAGG-TTCATCAGTTGA	19.7 kb
		stop codon	*954-956 (Exon 5)*		
		CFHR5	**NM_030787.4**		
		start codon	*110-112 (Exon 1)*		
328	07845-L07601	Exon 1	152-153	CATGGGTATCCA-CTGTTGGGGGAG	5.2 kb
427	07846-L16757	Exon 2	223-224	GATGAAGAAGAT-TATAACCCTTTT	1.1 kb
232 #	07847-L07603	Exon 3	414-415	ATCTTCAGGACT-AATACATCTGGA	437.8 kb
		stop codon	*1817-1819 (Exon 10)*		
433 ¬	06963-L06543	***CRB1***			3937.2 kb
355 ¬	07777-L07533	***TNNT2***			

§ Minor allele detected. This probe will only generate a signal when the C allele of rs1061170 (minor allele of the Y402H polymorphism) is present.
∞ Major allele detected. The presence of the minor allele will result in a decreased probe signal.
¬ Flanking probe. Included to facilitate the determination of the extent of a deletion/duplication. Copy number alterations of flanking and reference probes are unlikely to be related to the condition tested.
* We recommend to disregard results from this probe.
# This probe's specificity relies on a single nucleotide difference compared to a related gene. 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.

Note: Many probes are located in introns due to the presence of closely related sequences for many of the exon sequences. Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Table 2b. Chromosome 6

Length (nt)	SALSA MLPA probe	Gene[a] exon	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)
		C2	**NM_000063.6**	
		start codon	*37-39 (Exon 1)*	
208 §	07849-L07606	Exon 7 C allele of rs9332739	990-991	GATATGACTGAC-GTGATCAGCAGC
211 ∞	07849-L16761	Exon 7 G allele of rs9332739	990-991	GATATGACTGAG-GTGATCAGCAGC
		stop codon	*2293-2295 (Exon 19)*	

§ Minor allele detected. This probe will only generate a signal when the C allele of rs9332739 is present.
∞ Major allele detected. The presence of the minor allele will result in a decreased probe signal.

Table 2c. Chromosome 10

Length (nt)	SALSA MLPA probe	Gene[a] exon	Ligation site	Partial sequence[b] (24 nt adjacent to ligation site)
		ARMS2	**NM_001099667.3**	
		start codon	*76-78 (Exon 1)*	
154 ∞	07848-L07604	Exon 1 G allele of rs10490924	280-281	TGATCCCAGCTG-CTAAAATCCACA
157 §	07848-L16762	Exon 1 T allele of rs10490924	280-281	TGATCCCAGCTT-CTAAAATCCACA
		stop codon	*397-399 (Exon 2)*	

§ Minor allele detected. This probe will only generate a signal when the G allele of rs10490924 is present.
∞ Major allele detected. The presence of the minor allele will result in a decreased probe signal.

a) See above section on exon numbering for more information.

b) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Note: Exon numbering used here may differ from literature! Complete probe sequences are available on request: info@mlpa.com. Please notify us of any mistakes: info@mlpa.com.

Related SALSA MLPA probemixes

- P296 aHUS: Contains probes for the genes *CD46* and *CFI*.

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P236 Product history	
Version	*Modification*
A3	One probe has a change in length. No change in sequence detected. Two control fragments at 88 and 96 nt have been replaced and two new control fragments at 100 and 105 nt have been included.
A2	Eight probes have a small change in length. No change in sequence detected.
A1	First release.

Implemented changes in the product description
Version A3-01 – 28 November 2019 (02P) - Product description rewritten and adapted to a new template. - Various minor textual or layout changes. - Exon numbering: Updated text and comments. - Warning added to Table 1 and Table 2 for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene. - Ligation sites of the probes targeting the *CFH*, *CFHR5*, *C2*, *ARMS2* and *KCNT2* genes 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. - For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36). *Version 15 – 12 July 2016 (55)* - Chromosomal location of the CFH gene area corrected. - More information on atypical hemolytic uremic syndrome added on page 1. - Related probemix added on page 2. - References added on page 2. - Section on 'Result obtained with SALSA MLPA probemix P236 ARMD mix-1' rewritten. - Various minor textual changes. *Version 14 – 08 March 2016 (55)* - Product description adapted to a new product lot (lot number added, new picture included). - Various minor textual changes.

More information: www.mlpa.com; www.mlpa.eu	
	MRC-Holland bv; Willem Schoutenstraat 1 1057 DL, Amsterdam, The Netherlands
E-mail	info@mlpa.com (information & technical questions); order@mlpa.com (orders)
Phone	+31 888 657 200