Product Description

SALSA® MLPA® Probemix P110-C1 FCGR mix 1 & SALSA® MLPA® Probemix P111-C1 FCGR mix 2

To be used with the MLPA General Protocol.

Version 02
For complete product history see page 13.

These products are for basic research and intended for experienced MLPA users only! These probemixes enable 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 these products or this product description are highly appreciated.

Catalogue numbers

- **P110-025R:** SALSA® MLPA® Probemix P110 FCGR mix 1, 25 reactions
- **P110-050R:** SALSA® MLPA® Probemix P110 FCGR mix 1, 50 reactions
- **P110-100R:** SALSA® MLPA® Probemix P110 FCGR mix 1, 100 reactions

- **P111-025R:** SALSA® MLPA® Probemix P111 FCGR mix 2, 25 reactions
- **P111-050R:** SALSA® MLPA® Probemix P111 FCGR mix 2, 50 reactions
- **P111-100R:** SALSA® MLPA® Probemix P111 FCGR mix 2, 100 reactions

SALSA® MLPA® Probemix P110 FCGR mix 1 and SALSA® MLPA® Probemix P111 FCGR mix 2 (hereafter: P110 FCGR mix 1 and P111 FCGR mix 2) are to be used in combination with:

1. SALSA® MLPA® Reagent Kit (Cat. No: EK1-FAM, EK1-CY5, EK5-FAM, EK5-CY5, EK20-FAM),
2. Data analysis software Coffalyser.Net™ (Cat. No: n.a.)

P110 FCGR mix 1 and P111 FCGR mix 2 can be used in combination with:

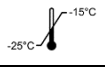
- SALSA® Reference Selection & Binning DNA SD038 (Cat. No: SD038)

Volumes and ingredients

Volumes			Ingredients
P110-025R P111-025R	**P110-050R P111-050R**	**P110-100R P111-100R**	
40 µl	80 µl	160 µl	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

The MLPA probemixes are 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 these products: 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).

Storage and handling

Recommended storage conditions		

A shelf life of until the expiry date is guaranteed, when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. These products should not be exposed to more than 25 freeze-thaw cycles. Do not use the products 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.

Certificate of Analysis
Information regarding 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 these products.

General information
SALSA® MLPA® Probemix P110 FCGR mix 1 and SALSA® MLPA® Probemix P111 FCGR mix 2 are **research use only (RUO)** assays for the detection of genomic rearrangements and point mutations in the 180 kb FCGR2/3 locus at 1q23.3.

Receptors for the Fc portion of IgG play an essential role in the protection of the organism against foreign antigens by removing antigen-antibody complexes from the circulation. Receptors are present on monocytes, macrophages, neutrophils, natural killer (NK) cells, and T and B lymphocytes. The receptors participate in diverse functions, such as phagocytosis of immune complexes and modulation of antibody production by B cells. Genes for several low-affinity Fc gamma receptors are clustered on chromosome 1q23.3 Within a 180 kb chromosomal area are genes for the FCGR2A, FCGR2B, FCGR2C, FCGR3A, and FCGR3B proteins. In addition, this region contains genes for the HSPA6 and HSPA7 heat shock proteins.

Due to high similarity between these *FCGR* genes and their close proximity, gene rearrangements are frequent in this chromosomal region. Various functionally relevant polymorphisms (SNPs) in these genes, as well as copy number variation of the *FCGR2C*, *FCGR3A,* and *FCGR3B* genes, have been reported. The MLPA probemixes P110 FCGR mix 1 and P111 FCGR mix 2 cover the mentioned *FCGR* genes and are intended to detect both copy number changes of these genes as well as frequent polymorphisms and point mutations, such as *FCGR2B* -386G/C, *FCGR2B* -120A/T, *FCGR3A* p.Val158Phe, *FCGR2B* p.Ile232Thr, *FCGR2A* p.His166Arg, and *FCGR2A* p.Gln62Trp. Probes for *FCGR3B* Human Neutrophil Antigen 1 (HNA1) alleles NA1, NA2 and SH, and *FCGR2C* STOP, classic and non-classic ORF haplotypes are also included.

These products are not CE/FDA registered for use in diagnostic procedures. The SALSA® MLPA® technique is covered by US patent 6,955,901 and corresponding patents outside the US. The purchase of these products includes a license to use only this amount of product solely for the purchaser's own use.

Gene structure and transcript variants:
Entrez Gene shows transcript variants of each gene: https://www.ncbi.nlm.nih.gov/gene
For NM_ mRNA reference sequences: https://www.ncbi.nlm.nih.gov/nuccore?db=nucleotide

Exon numbering
The *FCGR2A*, *FCGR2B,* and *FCGR2C* exon numbering used in this P110-C1 FCGR mix 1 and P111-C1 FCGR mix 2 product description is adopted from Nagelkerke et al. 2015. The *FCGR3A* and *FCGR3B* exon numbering is the exon numbering from the RefSeq transcript NM_000569.8 and NM_001271036.2, respectively. 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
P110-C1 FCGR mix 1 contains 40 MLPA probes with amplification products between 130 and 494 nucleotides (nt) (Table 1a). P111-C1 FCGR mix 2 contains 38 MLPA probes with amplification products between 130 and 490 nucleotides (nt) (Table 1b).

Both P110 FCGR mix 1 and P111 FCGR mix 2 contain 14 probes each for determination of copy number changes and genomic rearrangements (Table 2). Furthermore, P110 FCGR mix 1 also contains 18 mutation-

specific probes and P111 FCGR mix 2 contains 16 mutation-specific probes which will only generate a signal when the mutation is present (Table 3). In addition, for both probemixes eight 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).

These probemixes contain 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 MLPA (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

MLPA technique validation
Internal validation using 16 different DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample type or 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 peripheral blood, free from impurities known to affect MLPA reactions. MRC Holland has tested and can recommend the following extraction methods:

- QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual)
- Promega Wizard Genomic DNA Purification Kit (manual)
- Salting out (manual)

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. 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 different reference samples should be included in each MLPA experiment for data normalisation. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

Selecting suitable reference samples for the P110 FCGR mix 1 and P111 FCGR mix 2 probemixes is complicated due to the presence of mutation-specific probes in these probemixes. Suitable reference samples have a copy number of two for the target sequences of all reference probes and FCGR copy number probes, and a known copy number for the target sequences of the mutation-specific probes. SALSA® Reference Selection & Binning DNA SD038 can facilitate the identification of suitable reference DNA samples (see below).

Positive control DNA samples
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de) have diverse collections of biological resources which may be used as positive control

DNA samples in your MLPA experiments. The quality of cell lines can change; therefore deviations to the indicated copy number alteration (CNA) findings might occur.

SALSA® Reference Selection & Binning DNA SD038

The selection of suitable reference DNA samples that can be used with P110 FCGR mix 1 and P111 FCGR mix 2 is complicated. To facilitate the selection of suitable reference DNA samples from your own sample collection, a reference selection DNA sample is provided with these probemixes from MRC Holland. SALSA® Reference Selection & Binning DNA SD038 should only be used for initial experiments on DNA samples from healthy individuals with the intention to identify suitable reference samples. **SD038 should not be used as a reference sample in subsequent experiments.**

In addition, SALSA® Reference Selection & Binning DNA SD038 can also be used for binning of all mutation-specific probes that are included in P110 FCGR mix 1 and P111 FCGR mix 2 probemixes. Inclusion of one reaction of SD038 in initial MLPA experiments is essential as it can be used to aid in data binning of the peak pattern using Coffalyser.Net. 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). SD038 should never be used as a reference sample in the MLPA data analysis, neither should it be used in quantification of mutation signals. For further details, please consult the SD038 product description, available online: www.mrcholland.com. **These products are for research use only (RUO).**

Data analysis

Coffalyser.Net should be used for data analysis in combination with the appropriate lot-specific Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net is freely downloadable at www.mrcholland.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

Interpretation of results

The standard deviation of each individual reference probe (with exception of the mutation-specific probes) over all the reference samples should be ≤0.10 and the final ratio (FR) 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 FR of the probes can be used to interpret MLPA results:

Copy Number status: Probes with one copy in reference samples	Final ratio
No copies	FR = 0
One copy	0.80 < FR < 1.20
Two copies	1.65 < FR < 2.25
Ambiguous copy number	All other values

Copy Number status: Probes with two copies in reference samples	Final ratio
No copies	FR = 0
One copy	0.40 < FR < 0.65
Two copies	0.80 < FR < 1.20
Three copies	1.30 < FR < 1.65
Four copies	1.75 < FR < 2.15
Ambiguous copy number	All other values

For probes with a copy number of four in the reference samples, the expected normal copy numbers are two, three and four (see Table 3), corresponding to probe ratios of 0.5, 0.75 and 1, respectively. The probe ratios of probes detecting four copies in the reference samples should be interpreted together with the results of surrounding copy number probes.

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of Coffalyser.Net (Calculations, cut-offs and interpretation remain unchanged.) Please note that Coffalyser.Net also shows arbitrary borders as part of the statistical analysis of

results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. single nucleotide variants, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination in the DNA sample) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes are unlikely to have any relation to the condition tested for.
- False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: a lower injection voltage or a shorter injection time, or a reduced amount of sample by diluting PCR products.

P110 FCGR mix 1 and P111 FCGR mix 2 specific information

We recommend starting the analysis by establishing copy number changes and gene rearrangements with the use of only the copy number probes (Table 2). In this step it is essential to combine copy number results from both P110 and P111 (e.g. in Excel) and then sort these results according to chromosomal location to get a comprehensive view of the genomic arrangement of the loci.

Status of variants detected by mutation-specific probes (Table 3) can subsequently be established. For most mutations, two probes cover the alternative alleles, most often present as a single nucleotide change. These mutation-specific probe pairs also share the same or similar run length in the P110 and P111 probemixes (Table 3). To determine the copy number of specific alleles in separate homologous genes, the first important step is to control that the sum of copies of both alleles matches the sum of the genomic copies in the segments in which the nucleotide changes are present. Collect these copy numbers from the copy number probes (Table 2) designed at genomic locations closest to the mutation-specific probes.

The determination of copy numbers of specific alleles at different loci is best illustrated with an example: The 256 nt probe in P110 detects the -120A allele, while the probe at the same run length in P111 detects the -120T allele, both in *FCGR2B*. In addition, the 256 nt probe in P111 detects the T at the corresponding position in *FCGR2C*. Results show one copy of the A allele and four copies of the T allele, while *FCGR2B* and *FCGR2C* copy numbers in the corresponding gene segments have been determined to three and two, respectively. The total copies of the alleles match the total number of gene copies; both are five. In this example it is furthermore

likely that *FCGR2B* harbours one -120A and two -120T alleles, while the additional two T copies belong to *FCGR2C*, as an A allele in the promoter region of *FCGR2C* has not been reported.

Limitations of the procedure

- MLPA cannot detect any changes that lie outside the target sequence of the probes and will not detect copy number neutral inversions or translocations. Even when MLPA did not detect any aberrations, the possibility remains that biological changes in that gene or chromosomal region *do* exist but remain undetected.
- Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can cause false positive results. Mutations/SNVs (even when >20 nt from the probe ligation site) can reduce the probe signal by preventing ligation of the probe oligonucleotides or by destabilising the binding of a probe oligonucleotide to the sample DNA.

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 in sequence data 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.

Table 1a. P110-C1 FCGR mix 1

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]					
		Other	*FCGR2A*	*FCGR3A*	*FCGR2C*	*FCGR3B*	*FCGR2B*
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 19551-L26105	2p13					
137	**FCGR3A probe** 21806-L30537			Intron 1			
143	Reference probe 10113-L31635	8q22					
148	**FCGR2A/2C probe** 21814-L30545		**p.Leu273Pro**		Exon 8		
160	**FCGR3B probe** 21819-L30550					Intron 3	
166	**FCGR3A/3B probe** 21822-L30553			Exon 3		**p.Leu38= NA1**	
172	Reference probe 16647-L19180	10q23					
178	**HSPA7 probe** 21816-L30547	HSPA7					
184	**FCGR2B probe** 21824-L30555						**-386C**
190	**FCGR2A probe** 21799-L30530		**p.Val204_ Gln205insLeu**				
196	**FCGR3A probe** 21803-L30534			Exon 5			
202	**FCGR2C/2B probe** 21827-L31274				Exon 5		**p.Ile232**
211	**FCGR2C probe** 03609-L02976				**c.798+1A n.c. ORF1/2**		
220	**FCGR2C/2B probe** 21826-L30557				**c.392-20G>C ORF**		Intron 3
238 Ж	**FCGR2A/2C probe** 21813-SP1007-L30544		Intron 7		**c.799-1C>G ORF; n.c. ORF1**		
247	**FCGR2A probe** 21958-L30771		**p.Gln62**				
256	**FCGR2B probe** 21825-L30556						**-120A**
265	**FCGR2A probe** 21958-L30772		**p.Gln62Ter**				
274	**FCGR2A probe** 21795-L30526		Exon 2				
283	**FCGR2C probe** 21810-L30541				**p.Ter57; STOP**		
292	Reference probe 18491-L23716	3q12					
301	**FCGR3A probe** 21959-L30773			Exon 5			
319	**HSPA6 probe** 21802-L30533	HSPA6					
328	**FCGR2A probe** 21800-L30531		Intron 4				
337	**FCGR2B probe** 21828-L30559						Exon 8
346	**FCGR3B probe** 21821-L30552					**p.Asn65 NA1**	
355	**FCGR2A probe** 21797-L30528		**p.His166**				
364	**FCGR3A probe** 21804-L30535			Intron 4			
373	Reference probe 04278-L03682	12q12					
382	**FCGR3A/3B probe** 21820-L30551			Exon 3		**p.Val106Ile; NA2 and SH**	
392	**FCGR3A probe** 21866-L31482			**p.Val158Phe**			
400	**FCGR2C probe** 21809-L30540				**c.134-45T STOP**		
409	Reference probe 16934-L19877	4q12					
418	**FCGR2C probe** 21808-L30539				Upstream		
436	**FCGR2B probe** 21968-L30786						**p.Asn106del**
444	Reference probe 09077-L23425	19p13					
454	**FCGR2A probe** 21801-L30532		Intron 7				
463	**FCGR3A probe** 21807-L30538			Upstream			
474	**FCGR2C probe** 21815-L30546				Downstream		
494	Reference probe 19137-L26747	21q22					

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

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. When this occurs in reference samples, it can look like an increased signal in the test samples.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 1b. P111-C1 FCGR mix 2

Length (nt)	MLPA probe	Chromosomal position (hg18)[a]					
		Other	*FCGR2A*	*FCGR3A*	*FCGR2C*	*FCGR3B*	*FCGR2B*
64-105	Control fragments – see table in probemix content section for more information						
130	Reference probe 19551-L26105	2p13					
137	**FCGR3B probe** 21840-L30581					Exon 1	
142	**FCGR2A probe** 21841-L30582		Exon 1				
147	**FCGR2A/2C probe** 21842-L30583		Exon 8		**p.Pro280Leu**		
160	**FCGR3A probe** 21845-L30586			Intron 3			
166	**FCGR3B probe** 21846-L31114					**p.Leu38 NA2 and SH**	
172	Reference probe 16647-L19180	10q23					
178	**HSPA6 probe** 21847-L30588	HSPA6					
182	**FCGR2C/2B probe** 21848-L31275				Upstream		**-386G**
187	**FCGR2A probe** 21849-L30590		**c.739+871A**				
196	**FCGR3B probe** 21803-L30591					Exon 5	
203	**FCGR2B probe** 21851-L31575						**p.Ile232Thr**
209 Ж	**FCGR2A/2C probe** 21852-SP1009-L30594		Intron 7		**c.798+1A>G**		
219	**FCGR2C probe** 21853-L30595				**c.392-20G STOP**		
229	**FCGR3A probe** 21854-L30596			Downstream			
238	**FCGR2C probe** 21855-L30597				**c.799-1C n.c. ORF 2**		
247	**FCGR2A probe** 21856-L31576		**p.Gln62Trp**				
256	**FCGR2C/2B probe** 21857-L30599				Upstream		**-120T**
265	Reference probe 12434-L27286	14q24					
274	**FCGR2B probe** 21858-L30600						Exon 7
283	**FCGR2C/2B probe** 21859-L30601				**p.Ter57Gln ORF**		Exon 3
292	Reference probe 18491-L23716	3q12					
301	**FCGR3B probe** 21960-L30774					Exon 5	
320	**HSPA7 probe** 22377-L31573	HSPA7					
337	**FCGR3B probe** 21862-L30605					**p.Ala78Asp SH**	
346	**FCGR3A/3B probe** 21863-L30606			Exon 3		**p.Asn65Ser NA2 and SH**	
355 Δ	**FCGR2A probe** 04814-L10736		**p.His166Arg**				
364	**FCGR3B probe** 21864-L30607					Intron 4	
373	Reference probe 04278-L03682	12q12					
393	**FCGR3A/3B probe** 21866-L30609			**p.Val158**		Exon 4	
400	**FCGR2C/2B probe** 21867-L30610				**c.134-45 T>C ORF**		Intron 2
409	Reference probe 16934-L19877	4q12					
418	**FCGR2B probe** 21868-L30611						Upstream
444	Reference probe 09077-L23425	19p13					
454	**FCGR2C probe** 21870-L30613				Intron 7		
463	**FCGR3B probe** 21871-L30614					Upstream	
472	**FCGR2A probe** 21872-L30615		Downstream				
490	Reference probe 19137-L25693	21q22					

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

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. When this occurs in reference samples, it can look like an increased signal in the test samples.

Δ This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

The probe lengths in the table above may vary slightly depending on the capillary electrophoresis machine settings. Please see the most up-to-date Coffalyser sheet for exact probe lengths obtained at MRC Holland.

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

Table 2a. P110-C1/P111-C1 copy number probes arranged according to chromosomal location

Length (nt)		MLPA probe	Gene	Exon[a]	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe	Location ligation site (hg18)
P110	P111						
	142	21841-L30582	*FCGR2A*	Exon 1	TCACCCAGCAGC-AGCAAAACTGTC	0.5 kb	01-159.741.959
274		21795-L30526	*FCGR2A*	Exon 2	ACTCACCAGCTT-GACTGTCTGCAG	4.7 kb	01-159.742.416
328		21800-L30531	*FCGR2A*	Intron 4	GAATCTTGCATT-GGTGAGTGACTC	6.6 kb	01-159.747.105
454		21801-L30532	*FCGR2A*	Intron 7	GAGATCTTCAAC-CATTTCTTTTGA	2.5 kb	01-159.753.716
	472	21872-L30615	*FCGR2A*	Downstream	TGCCTTTCTGAC-AACTTGTGTTCC	4.1 kb	01-159.756.252
319		21802-L30533	*HSPA6*	Upstream	TCTGGCCATTCA-CTAAGGAACCAG	6.2 kb	01-159.760.390
	178	21847-L30588	*HSPA6*	Downstream	ACTGCTCCCTGA-TTTCATAGACCA	9.8 kb	01-159.766.552
	229	21854-L30596	*FCGR3A*	Downstream	GCTCTCTGTGGG-TTCGGGGGTTCC	2.0 kb	01-159.776.367
196		21803-L30534	*FCGR3A*	Exon 5	TCAAATCCTTCA-TCATGTCAGTTC	1.1 kb	01-159.778.363
301		21959-L30773	*FCGR3A*	Exon 5	AGACAAACATTC-GAAGCTCAACAA	0.3 kb	01-159.779.491
364		21804-L30535	*FCGR3A*	Intron 4	CACCAAACACTG-AGCAAAGGCTCC	2.1 kb	01-159.779.822
	160	21845-L30586	*FCGR3A*	Intron 3	TATTGCTCAGCC-TGGCAATTCGTG	4.0 kb	01-159.781.941
137		21806-L30537	*FCGR3A*	Intron 1	TGGATTGAGCTC-CTAGGACAAGCC	4.5 kb	01-159.785.975
463		21807-L30538	*FCGR3A*	Upstream	TAGGAATGAAAA-AGTGTTTAGTCA	13.6 kb	01-159.790.443
418		21808-L30539	*FCGR2C*	Upstream	CAAGTTAATAAT-AATGACATCTTT	31.3 kb	01-159.804.087
	454	21870-L30613	*FCGR2C*	Intron 7	GAGATCTTTAAG-CATTTCTTTTGA	2.5 kb	01-159.835.371
474		21815-L30546	*FCGR2C*	Downstream	GAACACAAGTTC-TCAGAAAGGCAA	4.1 kb	01-159.837.903
	320	22377-L31573	*HSPA7*	Upstream	TCTGGCCATTCC-TTAAGGAAACAG	6.1 kb	01-159.842.039
178		21816-L30547	*HSPA7*	Downstream	ACTGCTCCCTGT-TTTCATAGACCA	11.7 kb	01-159.848.156
	196	21803-L30591	*FCGR3B*	Exon 5	TCAAATCCTTCT-TCATGTCAGTTC	1.1 kb	01-159.859.800
	301	21960-L30774	*FCGR3B*	Exon 5	TGTTGAGCTTCA-AATGTTTGTCTT	0.3 kb	01-159.860.931
	364	21864-L30607	*FCGR3B*	Intron 4	GAGCCTTTGCTA-AGTGTTTGGTGA	2.1 kb	01-159.861.262
160		21819-L30550	*FCGR3B*	Intron 3	TAGTGCTCAGAG-TGGCAATTCGTG	3.9 kb	01-159.863.391
	137	21840-L30581	*FCGR3B*	Exon 1	TGGATTGAGCTA-CCAGGACAAGCC	4.5 kb	01-159.867.333
	463	21871-L30614	*FCGR3B*	Upstream	CACTAAACACTA-TTTCATTCCTAC	13.8 kb	01-159.871.803
	418	21868-L30611	*FCGR2B*	Upstream	GAGCCTTCTGAA-AGTGATGTGTCA	28.2 kb	01-159.885.573
	274	21858-L30600	*FCGR2B*	Exon 7	TTGTCAGCCTCA-TCAGGATTAGTG	0.1 kb	01-159.913.761
337		21828-L30559	*FCGR2B*	Exon 8	AATAGGTGATTG-TGTTCTCAGCCT		01-159.913.900

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

[b] Complete probe sequences are available at www.mrcholland.com. Please notify us of any mistakes: info@mrcholland.com.

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

Table 2b. P110-C1/P111-C1 mutation-specific probes arranged according to chromosomal location

Lenght (nt)		MLPA probe	Gene / Variant	Partial sequence[a] (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
P110	P111						
***FCGR2A*-p.Val204_Gln205insLeu** enhances binding of FcγRIIa to all subclasses of IgG and was found exclusively among carriers with the *FCGR2A* 166Arg/Arg genotype (Omar et al. 2012). The ***FCGR2A* c.739+871A>G** mutation caused retention and expression of exon 6 demonstrating enhanced cellular activation (van der Heijden et al. 2013). Probe detects the normal allele.							
190		21799-L30530	*FCGR2A* c.612_613insCTT; p.Val204_Gln205insLeu	ATCACTGTCCTT-CAAGGTATGGGG	0-2	01-159.746.485	rs150311303
	187	21849-L30590	*FCGR2A* c.739+871A	AAACCAGGTGAA-TACAGAGTTGTC	0-2	01-159.748.241	rs72717038
***FCGR2A*-p.Gln62Trp** (formerly known as p.Gln27Trp): The nucleotide polymorphism CA>TG (p.Gln62Trp) was first described in common variable immunodeficiency (CVID) and CVID-like patients (Flinsenberg et al. 2014) and has shown strong linkage disequilibrium (LD) with classic *FCGR2C* – ORF haplotype in Europeans (Nagelkerke et al. 2019). The *FCGR2A* c.184C>T polymorphism alone without a change in c.185 to G has not been found before (rs9427397); it would result in a change from glutamine (CAG) to stop (TAG) at position p.62. Such a change will be detected by the 265 nt probe in P110.							
247		21958-L30771	*FCGR2A* c.184C c.185A; p.Gln62	TCTGACAAGCCA-GGGGGCTCGCAG	0-2	01-159.742.828	rs201218628
	247	21856-L31576	*FCGR2A* c.184C>T c185A>G; p.Gln62Trp	GCGAGCCCCCCA-GCATGTCAGAGT	0-2	01-159.742.828	rs201218628

Lenght (nt)		MLPA probe	Gene / Variant	Partial sequence[a] (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
P110	P111						
265		21958-L30772	*FCGR2A* c.184C>T; p.Gln62Ter	TCTGACAAGCTA-GGGGGCTCGCAG	0-2	01-159.742.828	rs9427397
***FCGR2A*-p.His166Arg** (formerly known as p.His131Arg): A single nucleotide polymorphism results in a histidine or arginine at position 166. Histidine at this position has higher affinity for IgG1 and IgG2 in comparison with arginine and has been associated with Kawasaki disease (KD) in genome wide association studies (Khor et al. 2011), while an arginine instead has been associated with increased risk of developing systemic lupus erythematosus (SLE, Yuan et al. 2009).							
	355 Δ	04814-L10736	*FCGR2A* c.497A>G; p.His166Arg	TGGGATCCAAAC-GGGAGAATTTCT	0-2	01-159.746.369	rs1801274
355		21797-L30528	*FCGR2A* c.497A; p.His166	GAAATTCTCCCA-TTTGGATCCCAC	0-2	01-159.746.369	rs1801274
***FCGR2A*-p.Leu273Pro/*FCGR2C*-p.Pro280Leu:** *FCGR2A* c.818T and *FCGR2C* c.839C are homologous positions with one nucleotide difference in *FCGR2A* and *FCGR2C*, respectively. The c.818T>C nt polymorhism (p.Leu273Pro) is introduced to *FCGR2A* and c.839C>T (p.Pro280Leu) to *FCGR2C* in *FCGR2A/2C* and *FCGR2C/2A* chimeric genes, respectively (Nagelkerke et al. 2015).							
	147	21842-L30583	*FCGR2A* wt / *FCGR2C* c.839C>T; p.Pro280Leu	TGGTTTCTTCAA-GTTGTCTCTTTC	2-4	01-159.754.429/ 01-159.836.084	*FCGR2C*: rs867055986
148		21814-L30545	*FCGR2A* c.818T>C; p.Leu273Pro/*FCGR2C* wt	AAAGAGACAACC-TGAAGAAACCAA	2-4	01-159.754.429/ 01-159.836.084	*FCGR2A*: rs382627
***FCGR3A*-p.Val158Phe:** A single nucleotide polymorphism results in a valine or phenylalanine at position 158 (p.Val158Phe). Valine in this position has higher affinity for IgG1 and IgG3 compared to phenylalanine and has been found overrepresented in idiopathic thrombocytopenic purpura patients (Breunis et al. 2008, Carcao et al. 2003). Europeans homozygous for 158Val have a higher risk of developing rheumatoid arthritis (Lee et al. 2008).							
392		21866-L31482	*FCGR3A* c.526G>T; p.Val158Phe	GCAGGGGGCTTT-TTGGGAGTAAAA	0-2	01-159.781.166	rs396991
	393	21866-L30609	*FCGR3A* c.526G; p.Val158 / *FCGR3B* wt	GCAGGGGGCTTG-TTGGGAGTAAAA	2-4	01-159.781.166/ 01-159.862.610	rs396991
***FCGR3B* HNA1 variants:** The NA1, NA2 and SH haplotypes determine the allotypic variants of the Human Neutrophil Antigen 1 (HNA1), involved in allo-immunisation against neutrophilic granulocytes (Matsuo et al. 2000; Steffensen et al. 1999).							
382		21820-L30551	*FCGR3A* wt / *FCGR3B* c.316G>A; p.Val106Ile; NA2 and SH	TAGAAGTCCATA-TCGGTGAGTTGA	2-4	01-159.784.838/ 01-159.866.195	*FCGR3B*: rs2290834
	337	21862-L30605	*FCGR3B* c.233C>A; p.Ala78Asp; SH	CTTCATTGACGA-TGCCACAGTCAA	0-2	01-159.866.278	rs5030738
346		21821-L30552	*FCGR3B* c.194A; p.Asn65; NA1	TCACAATGAGAA-CCTCATCTCAAG	0-2	01-159.866.317	rs448740
	346	21863-L30606	*FCGR3A* wt / *FCGR3B* c.194A>G; p.Asn65Ser; NA2 and SH	TTGAGATGAGGC-TCTCATTGTGAA	2-4	01-159.784.961/ 01-159.866.317	*FCGR3B*: rs448740
166		21822-L30553	*FCGR3A* wt / *FCGR3B* c.114T>C; p.Leu38=; NA1	TACAGGTTGCTC-GAGAAGGACAGT	2-4	01-159.785.040/ 01-159.866.397	*FCGR3B*: rs527909462
	166	21846-L31114	*FCGR3B* c.114T; p.Leu38; NA2 and SH	CTGTCCTTTTCA-AGCACGCTGTAC	0-2	01-159.866.397	rs527909462
***FCGR2C* STOP/ORF haplotypes:** In most people, the *FCGR2C* gene is not expressed due to a stop codon at c.169 in exon 3 (*FCGR2C*-p.Ter57). The classic ORF haplotype consists of eight nucleotide changes in *FCGR2C* in intron 2, including *FCGR2C* c.134-45T>C, and exon 3, with the most important a *FCGR2C* c.169T>C polymorphism converting the stop codon into a glutamine and thereby enabling expression of the gene (*FCGR2C*-p.Ter57Gln) (Metes et al. 1998; Su et al. 2002). The *FCGR2C* c.392-20G>C alteration in intron 3 has also been shown linked to the ORF haplotype (Nagelkerke et al. 2015). In non-classical (n.c.) ORF1 and 2, presence of an A at the splice donor site c.798+1 result in splicing out of exon 7 and loss of expression due to a frameshift and activation of a novel stop codon in exon 8. In n.c. ORF 2, presence of C at 799-1 activates a cryptic acceptor splice site within intron 7 causing re-introduction of a 62 bp sequence of intron 7 into the transcript (van der Heijden et al. 2012). The expressed ORF haplotype has been associated with susceptibility to KD in Europeans and to idiopathic thrombocytopenic purpura (ITP) (Breunis et al. 2008; Nagelkerke et al. 2019).							
400		21809-L30540	*FCGR2C* c.134-45T STOP	GAGGAAGCCCAA-GAGCCTGAGAGG	0-2	01-159.825.931	rs549681560

Lenght (nt)		MLPA probe	Gene / Variant	Partial sequence[a] (24nt adjacent to ligation site)	Normal copy number	Location ligation site (hg18)	rs#
P110	P111						
	400	21867-L30610	*FCGR2C* c.134-45T>C ORF / *FCGR2B* wt	CTCTCAAGCTCC-TGGGCTTCCTCT	2-4	01-159.825.931/ 01-159.907.762	*FCGR2C*: rs549681560
283		21810-L30541	*FCGR2C* c.169T; p.Ter57;STOP	GTTGATCCACTA-GGGCTCGAGTTT	0-2	01-159.826.011	rs759550223
	283	21859-L30601	*FCGR2C* c.169T>C; p.Ter57Gln; ORF / *FCGR2B* wt	ACTCGAGCTCCA-GTGGATCAACGT	2-4	01-159.826.011/ 01-159.907.842	*FCGR2C*: rs759550223
	219	21853-L30595	*FCGR2C* c.392-20G STOP	CACAGAAAACCC-CAGAGGACCCGG	0-2	01-159.827.538	rs530707246
220		21826-L30557	*FCGR2C* c.392-20G>C ORF / *FCGR2B* wt	CGGGTCCTCTGC-GGTTTTTTGTGT	2-4	01-159.827.538/ 01-159.909.370	*FCGR2C*: rs530707246
	209 Ж	21852-SP1009-L30594	*FCGR2A* wt / *FCGR2C* c.798+1A>G	GGAGAGAAGGGA-CAAGGCAGGAAGAAAAGGAGATGGC TGGGATTACTCA**C**-CTCAAATTGGGC	2-4	01-159.750.347/ 01-159.832.005	*FCGR2C*: rs76277413
211		03609-L02976	*FCGR2C* c.798+1A n.c. ORF 1 and 2	CCCAATTTGAGA-TGAGTAATCCCA	0-2	01-159.832.005	rs76277413
238 Ж		21813-SP1007-L30544	*FCGR2A* wt / *FCGR2C* c.799-1C>G ORF; n.c. ORF 1	CGTCCAGGTGG**C**-TGCAGGAAAGCATTTAAAACCCATAG GATAATTCA-ATACACCGGGGA	2-4	01-159.754.388/ 01-159.836.043	*FCGR2C*: rs430178
	238	21855-L30597	*FCGR2C* c.799-1C n.c. ORF 2	GCTTTCCTGCAC-CCACCTGGACGT	0-2	01-159.836.043	rs430178
The ***FCGR2B*-p.Asn106del** variant abolishes binding of FcγRIIb to IgG1 (Jonsson et al. 2017). dbSNP also reports identical AAT deletions in corresponding sites in the homologous genes *FCGR2A* (rs760608327) and *FCGR2C* (rs765184850). In case an AAT deletion is detected, a long-range PCR is needed to identify which gene is affected.							
436		21968-L30786	*FCGR2B* c.316_318del, p.Asn106del	CTCCCCGCTGTC-GTTGTTGGCCTT	0-2	01-159.907.988	rs755222686
***FCGR2B/2C* -386G/C and -120T/A variants:** Two polymorphic sites in the *FCGR2B* and *FCGR2C* promoters form two haplotypes: 2B.1 (-386G/-120T) and 2B.2 (-386C/-120T). A third haplotype, 2B.4, is formed in *FCGR2B* only (-386C/-120A) (Su et al. 2004; Breunis et al. 2008; Tsang-A-Sjoe et al. 2016). The 2B.4 haplotype has been reported associated with SLE (Su et al. 2004; Blank et al. 2005).							
	182	21848-L31275	*FCGR2C* wt/ *FCGR2B* -386G	AAAGGGTGATGC-AGGACAGCGTGC	2-4	01-159.817.466/ 01-159.899.270	*FCGR2B*: rs3219018
184		21824-L30555	*FCGR2B* -386C	CACGCTGTCCTC-CATCACCCTTTC	0-2	01-159.899.270	rs3219018
	256	21857-L30599	*FCGR2C* wt/ *FCGR2B* -120T	AGTGAAAAAGAA-ATGTTCTGTTTT	2-4	01-159.817.732/ 01-159.899.536	*FCGR2B*: rs780467580
256		21825-L30556	*FCGR2B* -120A	AAACAGAACATA-TCTTTTTCACTT	0-2	01-159.899.536	rs780467580
***FCGR2B*-p.Ile232Thr:** A single nucleotide polymorphism results in an isoleucine or threonine at position 232 (p.Ile232Thr). The polymorphism affects downstream signalling and Ile232 provides stronger inhibitory signals compared to Thr232 (Li et al. 2003). Homozygosity for Thr232 protects against malaria, but the same allele is also strongly associated with susceptibility to SLE (Willcocks et al. 2010).							
	203	21851-L31575	*FCGR2B* c.695T>C; p.Ile232Thr	GGTCACTAGGAC-TGCTGTAGCGGC	0-2	01-159.910.422	rs1050501
202		21827-L31274	*FCGR2C* wt/ *FCGR2B* c.695T; p.Ile232	CCGCTACAGCAA-TCCCAGTGACCA	2-4	01-159.828.591/ 01-159.910.422	*FCGR2B*: rs1050501

[a] 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. When this occurs in reference samples, it can look like an increased signal in the test samples.

Δ This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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

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P110/P111 product history	
Version	*Modification*
C1	Probemix has been completely redesigned. New copy number probes, additional HNA allele probes and ORF/STOP haplotype probes have been added. Existing target probes have been redesigned, replaced or removed, and all reference probes have been replaced.
B2	Two reference probes have been replaced, one reference probe has been added, and one reference probe has been changed in length but not in sequence detected. In addition, the control fragments have been replaced (QDX2).
B1	First commercial release.

Implemented changes in the product description

Version C1/C1-02 – 17 April 2024 (05P)
- Product description rewritten and adapted to a new template.
- Product description adapted to the new SD version; SD038-S03.
- Publications added to the list of selected publications using P110 FCGR mix 1 and P111 FCGR mix 2.

Version C1/C1-01 — 17 June 2020 (02P)
- Product description completely rewritten and adapted to a new template.
- Product description adapted to new product versions (version numbers changed, changes in Tables).
- Both probemixes should be used with the SD038-S02 version.

Version 15 – 05 April 2017 (55)
- Product description adapted to a new lot (lot number added, new picture included).
- Minor textual and layout changes.

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