Product Description SALSA® MLPA® Probemix P469-A1 F5

To be used with the MLPA General Protocol.

Version A1.

Catalogue numbers:
- **P469-025R:** SALSA MLPA Probemix P469 F5, 25 reactions.
- **P469-050R:** SALSA MLPA Probemix P469 F5, 50 reactions.
- **P469-100R:** SALSA MLPA Probemix P469 F5, 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).

Certificate of Analysis: Information regarding storage conditions, quality tests, and a sample electropherogram from the current sales lot is available at 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.

General information: The SALSA MLPA Probemix P469 F5 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *F5* gene, which is associated with Factor V deficiency.

Mutations in the *F5* gene have been found to cause a rare bleeding disorder called factor V deficiency. These mutations prevent the production of functional coagulation factor V or significantly reduce the amount of the protein in the bloodstream. People with this condition typically have less than 10 percent of normal levels of coagulation factor V in their blood; the most severely affected individuals have less than 1 percent. A reduced amount of functional factor V prevents blood from clotting normally, causing episodes of abnormal bleeding that can be severe. Factor V deficiency results from mutations in both copies of the *F5* gene, although some people with a mutation in a single copy of the gene have mild bleeding problems. Heterozygous defects in the *F5* gene are also associated with two- to threefold increase in relative risk for pregnancy loss, and possibly other pregnancy complications such as preeclampsia, fetal growth retardation, and placental abruption.

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 *F5* exon numbering used in this P469-A1 F5 product description is the exon numbering from the RefSeq transcript NM_000130.4, which is identical to the LRG_553 sequence. The exon numbering and NM_ sequence used have been retrieved on 05/2020. 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 P469-A1 F5 contains 35 MLPA probes with amplification products between 130 and 429 nucleotides (nt). This includes 26 probes for the *F5* gene, one probe for each exon of the gene, with the exception of exon 2, and three probes for exon 25. In addition, nine reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.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 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 unrelated individuals who are from families without a history of Factor V deficiency. 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. 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: The standard deviation of each individual 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

- 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. 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 *F5* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P469 F5.
- 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.

***F5* mutation database:** https://databases.lovd.nl/shared/genes/F5. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). 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 *F5* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P469-A1 F5

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*F5*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
137	**F5 probe** 20743-L28793		**Exon 17**
142	**F5 probe** 20744-L28645		**Exon 3**
148	Reference probe 17232-L20568	5q14	
154	**F5 probe** 20745-L28646		**Exon 10**
159	**F5 probe** 20746-L28647		**Exon 8**
166	**F5 probe** 20747-L28648		**Exon 22**
172	**F5 probe** 20748-L28649		**Exon 5**
182	Reference probe 08731-L23593	9q21	
190	**F5 probe** 20749-L28650		**Exon 24**
196	**F5 probe** 20750-L28651		**Exon 18**
202	**F5 probe** 20751-L28652		**Exon 12**
208	**F5 probe** 20752-L28653		**Exon 1**
226	**F5 probe** 20754-L28656		**Exon 25**
232	**F5 probe** 20755-L28657		**Exon 21**
238	**F5 probe** 20756-L28658		**Exon 19**
244	**F5 probe** 20757-L28659		**Exon 13**
250	**F5 probe** 20758-L28794		**Exon 11**
257	Reference probe 10692-L11274	6p12	
264	**F5 probe** 20759-L28661		**Exon 23**
274	**F5 probe** 20760-L28662		**Exon 20**
282	Reference probe 05960-L21637	7p11	
292	**F5 probe** 20761-L28663		**Exon 14**
301	**F5 probe** 20762-L28664		**Exon 6**
310	**F5 probe** 20763-L28665		**Exon 16**
319	Reference probe 16274-L18566	20q11	
327	**F5 probe** 20764-L28666		**Exon 9**
335	**F5 probe** 20765-L28667		**Exon 15**
346	**F5 probe** 20766-L28668		**Exon 7**
355	Reference probe 00547-L00116	11q22	
372	**F5 probe** 20767-L28669		**Exon 25**
382	**F5 probe** 20768-L28670		**Exon 4**
400	Reference probe 01237-L00568	10p14	
418	**F5 probe** 20770-L28672		**Exon 25**
429	Reference probe 12456-L23201	22q12	

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

Table 2. *F5* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*F5* exon[a]	Ligation site NM_000130.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*146-148 (exon 1)*		
208	20752-L28653	Exon 1	224-225	GCCAAGGGACAG-AAGCGGCACAGC	14.0 kb
	No probe	Exon 2			
142	20744-L28645	Exon 3	425-426	TATATGCTGAAG-TCGGAGACATCA	11.6 kb
382	20768-L28670	Exon 4	619-620	AGTGAGGACAGT-GGACCCACCCAT	1.5 kb
172	20748-L28649	Exon 5	816-817	AAGCAAGAGCTG-GAGCCAGTCATC	2.5 kb
301	20762-L28664	Exon 6	1079-1080	TATCTTCTCTCA-CCCCAAAACATT	1.3 kb
346	20766-L28668	Exon 7	1101-1102	TAACTCAGCTGG-GATGCAGGCTTA	2.8 kb
159	20746-L28647	Exon 8	1416-1417	TCCTATTATCAG-AGCCCAGGTCAG	1.9 kb
327	20764-L28666	Exon 9	1476-1477	CCGCCCCTATAG-CATTTACCCTCA	0.7 kb
154	20745-L28646	Exon 10	1560-1561	CAACACCATGAT-CAGAGCAGTTCA	3.4 kb
250	20758-L28794	Exon 11	1779-1780	CATCGAACAGCA-GGCTGTGTTTGC	2.1 kb
202	20751-L28652	Exon 12	1981-1980 reverse	CCCACACTACAG-AAGTGCCACTGG	1.5 kb
244	20757-L28659	Exon 13	2323-2324	GACTATGATTAC-CAGAACAGACTG	6.4 kb
292	20761-L28663	Exon 14	5101-5102	AGAGCTGAAGTG-GATGATGTTATC	5.6 kb
335	20765-L28667	Exon 15	5233-5234	TGGTTTAAGGAA-GATAATGCTGTT	1.2 kb
310	20763-L28665	Exon 16	5497-5496 reverse	GACTTCTTTTCA-TAGTACCAGCTC	1.8 kb
137	20743-L28793	Exon 17	88 nt after exon 17	TTCCTTGTGCAT-ATACTTCCTAAG	1.9 kb
196	20750-L28651	Exon 18	10 nt after exon 18	AGGTATCACAAG-AGCCATGTGATA	1.0 kb
238	20756-L28658	Exon 19	5895-5896	AAGCACTGGTAT-CATATCTGATTC	1.0 kb
274	20760-L28662	Exon 20	5966-5965 reverse	AGATCCACCATT-GTTTAATCTTGC	0.6 kb
232	20755-L28657	Exon 21	6124-6125	ACCACAGAGTTC-TATGTAGCTTAC	2.7 kb
166	20747-L28648	Exon 22	6269-6270	CTAGATATATTA-GGATCTCTCCAA	2.1 kb
264	20759-L28661	Exon 23	6352-6353	TGTTCCACACCC-CTGGGTATGGAA	3.1 kb
190	20749-L28650	Exon 24	6670-6671	TCCATGGTGGAC-AAGGTAGAGTGG	1.2 kb
372	20767-L28669	Exon 25	6872-6873	CCTCAAACCATT-TAGAATGGGCAA	0.6 kb
226	20754-L28656	Exon 25	7444-7445	GGTGGTCATTAG-ATATGGAACAGA	0.8 kb
418	20770-L28672	Exon 25	8204-8205	GACAGGGCTTTG-CTTTTGCTGCAT	
		stop codon	*6818-6820 (exon 25)*		

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.

Related SALSA MLPA probemixes

P178 F8	Contains probes for the *F8* gene.
P207 F9	Contains probes for the *F9* and *F7* genes.
P243 SERPING1-F12	Contains probes for the *SERPING1* and *F12* genes.
P440 F10-F11	Contains probes for the *F10* and *F11* genes.

References

- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
- Schwartz M et al. (2007). Deletion of exon 16 of the dystrophin gene is not associated with disease. *Hum Mutat.* 28:205.
- Varga RE et al. (2012). MLPA-based evidence for sequence gain: pitfalls in confirmation and necessity for exclusion of false positives. *Anal Biochem.* 421:799-801.

P469 Product history	
Version	*Modification*
A1	First release.

Implemented changes in the product description
Version A1-01 — 28 May 2020 (02P) - Product description rewritten and adapted to a new template. *Version 01 – 13 January 2017 (55)* - Not applicable, new document.

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