Product Description
SALSA® MLPA® Probemix P440-A2 F10 + F11

To be used with the MLPA General Protocol.

Version A2
For complete product history see page 7.

Catalogue numbers:
- **P440-025R:** SALSA MLPA Probemix P440 F10 + F11, 25 reactions.
- **P440-050R:** SALSA MLPA Probemix P440 F10 + F11, 50 reactions.
- **P440-100R:** SALSA MLPA Probemix P440 F10 + F11, 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.mrcholland.com).

Certificate of Analysis
Information regarding storage conditions, 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 this product.

General information
The SALSA MLPA Probemix P440 F10 + F11 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *F10* and *F11* genes, which are associated with Rosenthal syndrome.

Factor X deficiency and Factor XI deficiency (Rosenthal syndrome) are two types of inherited autosomal recessive bleeding disorders characterised by haemorrhagic conditions of variable severity. Defects in the *F10* gene on chromosome 13q34 are the main cause of Factor X deficiency. The vitamin K-dependent protein encoded by this gene is coagulation factor X. Defects in the *F11* gene on chromosome 4q35.2 are the main cause of Factor XI deficiency. The protein encoded by this gene is coagulation factor XI.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK447152/.

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 *F10* exon numbering used in this P440-A2 F10 + F11 product description is the exon numbering from the LRG_548 sequence. The *F11* exon numbering used in this P440-A2 F10 + F11 product description is the exon numbering from the LRG_583 sequence. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG sequences. 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

The SALSA MLPA Probemix P440-A2 F10 + F11 contains 35 MLPA probes with amplification products between 166 and 454 nucleotides (nt). This includes nine probes for the *F10* gene (one probe for each exon and two probes for exon 1) and 16 probes for the *F11* gene (one probe for each exon and two probes for exon 1). In addition, ten 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).

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.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 the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.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 different unrelated individuals who are from families without a history of Rosenthal syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

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. Sample ID numbers NA03089, NA03330, NA05832, NA03887, NA06312, NA08254, NA00501, NA10313, and NA03013 from the Coriell Institute have been tested with this P440-A2 probemix at MRC Holland and can be used as positive control samples to detect heterozygous deletion/duplication of the entire *F10/F11* gene. The quality of cell lines can change; therefore samples should be validated before use.

Sample name	Source	Altered target genes in P440-A2	Expected copy number alteration
NA03089	Coriell Institute	*F10*	Heterozygous duplication
NA03330	Coriell Institute	*F10*	Heterozygous duplication
NA05832	Coriell Institute	*F10*	Heterozygous duplication
NA03887	Coriell Institute	*F10*	Heterozygous deletion
NA06312	Coriell Institute	*F10*	Heterozygous deletion
NA08254	Coriell Institute	*F10*	Heterozygous deletion
NA00501	Coriell Institute	*F11*	Heterozygous duplication
NA10313	Coriell Institute	*F11*	Heterozygous duplication
NA03013	Coriell Institute	*F11*	Heterozygous deletion

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.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 probe 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 for autosomal chromosomes or pseudo-autosomal regions:

Copy number status	Final ratio (FR)
Normal	0.80 < FR < 1.20
Homozygous deletion	FR = 0
Heterozygous deletion	0.40 < FR < 0.65
Heterozygous duplication	1.30 < FR < 1.65
Heterozygous triplication/homozygous duplication	1.75 < FR < 2.15
Ambiguous copy number	All other values

Note: The term “dosage quotient”, used in older product description versions, has been replaced by “final ratio” to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software 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. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region or in or near the *F10* gene. 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.
- 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.

Limitations of the procedure

- In most populations, the major cause of genetic defects in the *F10* and *F11* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P440 F10 + F11.
- 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 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.

***F10* and *F11* mutation databases**

https://dbs.eahad.org/. We strongly encourage users to deposit positive results in the EAHAD Coagulation Factor Variant Database. 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 SNVs and unusual results (e.g., a duplication of *F11* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

Table 1. SALSA MLPA Probemix P440-A2 F10 + F11

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]		
		Reference	*F10*	*F11*
64-105	Control fragments – see table in probemix content section for more information			
166	Reference probe 08222-L24146	10q		
172	**F10 probe** 19077-L25728		**Exon 1**	
178	**F11 probe** 19078-L24965			**Exon 15**
184	**F10 probe** 19079-L24966		**Exon 2**	
190	Reference probe 16424-L26299	18q		
196	**F11 probe** 19080-L24967			**Exon 11**
202	**F10 probe** 19081-L24968		**Exon 1**	
213	**F11 probe** 19083-L24970			**Exon 6**
220	Reference probe 12427-L13428	22q		
230 Ж	**F11 probe** 19084-SP0742-L24971			**Exon 2**
238 «	**F10 probe** 19348-L24984		**Exon 5**	
247	**F11 probe** 19086-L24973			**Exon 14**
256	Reference probe 16221-L18853	1q		
265	**F11 probe** 19087-L24974			**Exon 10**
274 «	**F10 probe** 19088-L24975		**Exon 4**	
283	**F11 probe** 19089-L25569			**Exon 5**
292	Reference probe 15724-L17704	12q		
301	**F11 probe** 19513-L26006			**Exon 1**
311	**F11 probe** 19091-L24978			**Exon 9**
319 «	**F10 probe** 19092-L24979		**Exon 7**	
330	Reference probe 08741-L08752	9q		
338	**F11 probe** 19093-L24980			**Exon 7**
344 «	**F10 probe** 19094-L24981		**Exon 8**	
355	**F11 probe** 19095-L25570			**Exon 8**
364	Reference probe 18296-L23077	8p		
373	**F11 probe** 19096-L24983			**Exon 13**
384 «	**F10 probe** 19515-L26008		**Exon 6**	
393	**F11 probe** 19098-L24985			**Exon 1**
400	Reference probe 14204-L15818	11p		
409	**F11 probe** 19099-L24986			**Exon 3**
418 Ж	**F11 probe** 19100-SP0744-L24987			**Exon 12**
427	Reference probe 21727-L30385	15q		
436 Ж	**F10 probe** 19101-SP0745-L24988		**Exon 3**	
445	**F11 probe** 19102-L24989			**Exon 4**
454	Reference probe 15515-L17370	7q		

[a] See section Exon numbering on page 1 for more information.

« 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.

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.

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

Table 2. P440-A2 probes arranged according to chromosomal location

Table 2a. *F11*

Length (nt)	SALSA MLPA probe	*F11* exon[a]	Ligation site NM_000128.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*109-111 (Exon 2)*		
301	19513-L26006	Exon 1	61 nt before exon 1, reverse	CTCTCTCAGTAC-AGCATATTTGTG	0.2 kb
393	19098-L24985	Exon 1	96-97	GTAAGCCAACAA-GGTCTTTTCAGG	0.9 kb
230 Ж	19084-SP0742-L24971	Exon 2	117-118 and 162-163	AGGATGATTTTC-45 nt spanning oligo-GGTAAGTAGAGT	4.5 kb
409	19099-L24986	Exon 3	6 nt before exon 3	CCATGTACTACA-TCACAGAATGTG	1.5 kb
445	19102-L24989	Exon 4	379-380	CAAGAGTGAATA-GGACAGCAGCGA	1.0 kb
283	19089-L25569	Exon 5	441-442	CCAGCTTGCAAC-AAAGACATTTAT	1.8 kb
213	19083-L24970	Exon 6	681-682	TTTTCACTGAAA-TCCTGTGCACTT	0.3 kb
338	19093-L24980	Exon 7	33 nt before exon 7	ATAGCTGGTGAA-TTGAGTCCCTGA	3.9 kb
355	19095-L25570	Exon 8	958-959	TACAAAGCTGCA-GGCACAGCATCC	0.1 kb
311	19091-L24978	Exon 9	19 nt before exon 9	GTCTCACTCTGA-CATGTGGTCTGC	0.4 kb
265	19087-L24974	Exon 10	1227-1228	ACATTAAGGTTG-TGTAAAATGGAT	3.5 kb
196	19080-L24967	Exon 11	1244-1245	TTCTGTTGCAGA-GTGTACCACCAA	1.6 kb
418 Ж	19100-SP0744-L24987	Exon 12	1467-1468 and 1521-1522	AATCAATCTGAA-54 nt spanning oligo-TATAAAATGGCA	0.8 kb
373	19096-L24983	Exon 13	1676-1677	GGGGTACAGAAA-ACTAAGAGGTAA	1.3 kb
247	19086-L24973	Exon 14	1776-1777	AAAATAACCCAT-AAGATGATCTGT	1.4 kb
178	19078-L24965	Exon 15	2585-2586	AATGTGAAGATA-ACAGAATTTCTG	
		stop codon	*1984-1986 (Exon 15)*		

Table 2b. *F10*

Length (nt)	SALSA MLPA probe	*F10* exon[a]	Ligation site NM_000504.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*58-60 (Exon 1)*		
172	19077-L25728	Exon 1	48-49	GACACAGTACTC-GGCCACACCATG	0.1 kb
202	19081-L24968	Exon 1	22 nt after exon 1	TCGCCCTTCAGA-CCCAAAAGCAGC	6.6 kb
184	19079-L24966	Exon 2	246-247	GAGACCTGCTCA-TACGAAGAGGCC	8.9 kb
436 Ж	19101-SP0745-L24988	Exon 3	8 nt and 42 nt after exon 3	AAGGTCAGTATT-34 nt spanning oligo-CAGGATATTTGA	1.0 kb
274 «	19088-L24975	Exon 4	388-389	AATACACCTGCA-CCTGTTTAGAAG	1.6 kb
238 «	19348-L24984	Exon 5	1 nt after exon 5	CATTCCCACAGG-TAGGAGGCACGT	2.8 kb
384 «	19515-L26008	Exon 6	571-570, reverse	CTGTTTCCCACA-GGGGTAGGGCCC	3.6 kb
319 «	19092-L24979	Exon 7	867-868	AGCGAGTTCTAC-ATCCTAACGGCA	1.8 kb
344 «	19094-L24981	Exon 8	1255-1256	TCATCATCACCC-AGAACATGTTCT	
		stop codon	*1522-1524 (Exon 8)*		

[a] See section Exon numbering on page 1 for more information.

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

« 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.

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.

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

Related SALSA MLPA probemixes

P011-B4 / P012-B4 VWF	Contain probes for the *VWF* gene involved in von Willebrand disease.
P178 F8	Contains probes for the *F8* gene involved in Haemophilia A.
P207 F9	Contains probes for the *F7*, *F8*, and *F9* genes involved in Factor IX deficiency/ Christmas disease.
P243 SERPING1 – F12	Contains probes for the *SERPING1* and *F12* genes.
P469 F5	Contains probes for the *F5* gene.

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.

Selected publications using SALSA MLPA Probemix P440 F10 + F11

- Chikasawa Y et al. (2014). Factor X M402T: a homozygous missense mutation identified as the cause of cross-reacting material-reduced deficiency. *Int J Hematol*. 100:345-352.
- Esteban J et al. (2017). High incidence of FXI deficiency in a Spanish town caused by 11 different mutations and the first duplication of F11: Results from the Yecla study. *Haemophilia*. 23:e488-e496.
- Girolami A et al. (2016). A family with factor X deficiency from Argentina: a compound heterozygosis because of the combination of a new mutation (Gln138Arg) with an already known one (Glu350Lys). B*lood Coagul Fibrinolysis.* 27:732-736.
- Mitchell M et al. (2019). Genotype analysis and identification of novel mutations in a multicentre cohort of patients with hereditary factor X deficiency. *Blood Coagul Fibrinolysis.* 30:34-41.
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- Pavlova A et al. (2015). Congenital combined deficiency of coagulation factors VII and X–different genetic mechanisms. *Haemophilia*. 21:386-391.
- Rath M et al. (2015). Large deletions play a minor but essential role in congenital coagulation factor VII and X deficiencies. *Hämostaseologie*. 35:S36-S42.
- Van Laer C et al. (2021). F11 Gene Duplication Causes Elevated FXI Plasma Levels and Is a Risk for Venous Thrombosis." *Thromb haemost*.

P440 product history	
Version	*Modification*
A2	Three reference probes have been removed and two reference probes have been replaced.
A1	First release.

Implemented changes in the product description
Version A2-02 – 05 April 2022 (04P) - Product description rewritten and adapted to a new template. - Ligation sites of the probes targeting the *F11* gene updated according to new version of the NM_ reference sequence. The NM_ reference sequence of *F10* was updated according to a new version. Ligation sites remained the same. - Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products. Version A2-01 — 05 September 2019 (02P) - Product description rewritten and adapted to a new template. - Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).

More information: www.mrcholland.com; www.mrcholland.eu	
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