

Product Description SALSA® MLPA® Probemix P178-B4 F8

To be used with the MLPA General Protocol.

Version B4. As compared to version B3, one reference probe has been replaced. For complete product history see page 8.

Catalogue numbers:

- **P178-025R:** SALSA MLPA Probemix P178 F8, 25 reactions.
- **P178-050R:** SALSA MLPA Probemix P178 F8, 50 reactions.
- **P178-100R:** SALSA MLPA Probemix P178 F8, 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 P178 F8 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *F8* gene, which is associated with haemophilia A.

Haemophilia A, one of the most common coagulation disorders, is caused by complete or partial deficiency in factor VIII clotting activity. It is an X-linked disease with a prevalence of ~1:5,000 live male births that is caused by mutations in the *F8* gene encoding factor VIII.

The *F8* gene is located on Xq28 and spans ~187 kb of genomic DNA. Deletions and duplications in the *F8* gene are reported in up to ~5% of the haemophilia A patients (Kim et al. 2012; Lannoy et al. 2012; Vencesla et al. 2012). The most common genetic defects in the *F8* gene are intron 22 inversions and intron 1 inversions, which occur in 43-45% and 2-5% of severe haemophilia A cases, respectively. These inversions cannot be detected by SALSA MLPA probemix P178 F8.

More information is available at <https://www.ncbi.nlm.nih.gov/books/NBK1404/>.

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 *F8* exon numbering used in this P178-B4 F8 product description is the exon numbering from the RefSeq transcript NM_000132.3, which is identical to the NG_011403.1 sequence. The exon numbering and NM_ sequence used have been retrieved on 11/2019. 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 P178-B4 F8 contains 43 MLPA probes with amplification products between 136 and 477 nucleotides (nt). This includes 33 probes for the *F8* gene: one probe for each exon, two probes for exon 1, 3, 7, 12 and 26, and three probes for exon 14. In addition, ten reference

probes are included that detect locations on the X-chromosome. 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 of the same sex 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 haemophilia A. 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 NA02325 from the Coriell Institute has been tested with this P178-B4 probemix at MRC-Holland and can be used as a positive control sample to detect a duplication of *F8* exon 1-22. 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:

Copy Number status: Male samples	Dosage quotient
Normal	$0.80 < DQ < 1.20$
Homozygous deletion	$DQ = 0$
Duplication	$1.65 < DQ < 2.25$
Ambiguous copy number	All other values

Copy Number status: Female samples	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 or in or near the *F8* 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.
- 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 causes of genetic defects in the *F8* gene are intron 22 inversions, intron 1 inversions and small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P178 F8.
- 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.

Factor VIII gene (*F8*) variant database: <http://f8-db.eahad.org/>. We strongly encourage users to deposit positive results in the factor VIII gene (*F8*) 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 SNPs and unusual results (e.g., a duplication of *F8* exons 8 and 10 but not exon 9) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P178-B4 F8

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18) ^a Reference	F8
64-105	Control fragments – see table in probemix content section for more information		
136	Reference probe 07103-L14743	Xp22	
142	F8 probe 05610-L05060		Exon 1
148	F8 probe 05618-L05068		Exon 7
154	F8 probe 05627-L06055		Exon 14
160 «	F8 probe 05633-L05083		Exon 20
166 *	Reference probe 04423-L05579	Xp22	
172	F8 probe 05612-L05062		Exon 2
179	F8 probe 05619-L14947		Exon 8
186	F8 probe 06290-L17545		Exon 6
191	F8 probe 05626-L17546		Exon 14
197 «	F8 probe 05634-L17547		Exon 21
202	Reference probe 13203-L14524	Xq13	
209	F8 probe 05611-L17548		Exon 1
215	F8 probe 05620-L17549		Exon 9
221	F8 probe 06288-L05892		Exon 5
229	F8 probe 05641-L05091		Exon 26
238	Reference probe 06910-L06490	Xq22	
247	F8 probe 05617-L05067		Exon 7
256	F8 probe 05621-L05071		Exon 10
265	F8 probe 05628-L05078		Exon 15
274	F8 probe 07044-L05899		Exon 12
292	Reference probe 02920-L02314	Xp21	
298	F8 probe 05614-L05891		Exon 4
305	F8 probe 06287-L06058		Exon 11
313	F8 probe 06506-L06340		Exon 3
319	F8 probe 05629-L05079		Exon 16
328	F8 probe 05637-L05087		Exon 23
346	Reference probe 05125-L04515	Xq26	
355	F8 probe 05623-L05893		Exon 12
364	F8 probe 05630-L05080		Exon 17
372	F8 probe 14164-L15766		Exon 24
382	Reference probe 02908-L02302	Xq22	
391 «	F8 probe 07045-L05898		Exon 22
402	F8 probe 05624-L05074		Exon 13
409	F8 probe 05631-L05081		Exon 18
418	F8 probe 13707-L15181		Exon 25
426	Reference probe 13207-L14528	Xp11	
434	Reference probe 13118-L15558	Xq21	
444	F8 probe 05613-L14948		Exon 3
452	F8 probe 05625-L14949		Exon 14
459 «	F8 probe 05632-L14950		Exon 19
468	F8 probe 13708-L15182		Exon 26
477	Reference probe 01391-L01039	Xp21	

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

* New in version B4.

« 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.

Table 2. F8 probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	F8 exon ^a	Ligation site NM_000132.3	Partial sequence ^b (24 nt adjacent to ligation site)	Distance to next probe
		<i>start codon</i>	172-174 (Exon 1)		
142	05610-L05060	Exon 1	24-25	GCACATCCAGTG-GGTTAAAGTTCCT	0.3 kb
209	05611-L17548	Exon 1	294-295	AGTGATCTCGGT-GAGCTGCCTGTG	22.9 kb
172	05612-L05062	Exon 2	346-347	AATCTTTCCAT-TCAACACCTCAG	2.5 kb
444	05613-L14948	Exon 3	462-463	ACCATCCAGGCT-GAGGTTTATGAT	0.1 kb
313	06506-L06340	Exon 3	538-537 reverse	TTTCCAGTAGGA-TACACCAACAGC	4.0 kb
298	05614-L05891	Exon 4	689-690	CCCCTGTGCCT-TACCTACTCATA	5.7 kb
221	06288-L05892	Exon 5	779-780	TTCAGGGAGTCT-GGCCAAGGAAAA	2.5 kb
186	06290-L17545	Exon 6	891-892	CAGGATAGGGAT-GCTGCATCTGCT	15.2 kb
247	05617-L05067	Exon 7	984-985	CACAGGAAATCA-GTCTATTGGCAT	0.2 kb
148	05618-L05068	Exon 7	1132-1133	CACTCTTGATGG-ACCTTGGACAGT	2.7 kb
179	05619-L14947	Exon 8	1213-1214	AAGTAGACAGCT-GTCCAGAGGAAC	0.6 kb
215	05620-L17549	Exon 9	1566-1567	CAGCATGAATCA-GGAATCTTGGGA	4.9 kb
256	05621-L05071	Exon 10	1659-1660	AACATCTACCCT-CACGGAATCACT	4.1 kb
305	06287-L06058	Exon 11	1830-1831	CGCTATTACTCT-AGTTTCGTTAAT	3.1 kb
355	05623-L05893	Exon 12	1975-1976	TTGATGAGAACC-GAAGCTGGTACC	0.1 kb
274	07044-L05899	Exon 12	2063-2062 reverse	TGTGCATGATGT-TGGAGGCTTGGGA	6.1 kb
402	05624-L05074	Exon 13	2163-2164	ATTGGAGCACAG-ACTGACTTCCTT	16.5 kb
452	05625-L14949	Exon 14	2655-2656	TCTGATCTCCAA-GAAGCCAAATAT	0.9 kb
191	05626-L17546	Exon 14	3577-3578	ATGGAAAGAACT-CTCTGAACTCTG	1.5 kb
154	05627-L06055	Exon 14	5111-5112	CACCTGGGCAAA-GCAAGGTAGGAC	22.3 kb
265	05628-L05078	Exon 15	5431-5432	AGAAAGTTGTTT-TCCAGGAATTTA	1.6 kb
319	05629-L05079	Exon 16	5587-5588	ATTCCTTCTATT-CTAGCCTTATTT	0.5 kb
364	05630-L05080	Exon 17	5818-5819	CTAACACACTGA-ACCCTGCTCATG	0.5 kb
409	05631-L05081	Exon 18	6073-6074	TCAGCATGGGCA-GCAATGAAAACA	1.9 kb
459 <	05632-L14950	Exon 19	6226-6227	GGCGGGTGAAT-GCCTTATTGGCG	0.7 kb
160 <<	05633-L05083	Exon 20	6308-6309	TCCCCTGGGAAT-GGCTTCTGGACA	1.5 kb
197 <<	05634-L17547	Exon 21	6405-6406	TCCGGATCAATC-AATGCCTGGAGC	3.8 kb
391 <<	07045-L05898	Exon 22	6572-6571 reverse	AATTTCTCGAT-AAGTCTGCCACT	32.9 kb
328	05637-L05087	Exon 23	6637-6638	CTGGGATAAAAC-ACAATATTTTTA	1.4 kb
372	14164-L15766	Exon 24	6782-6783	GAGTAAAGCAAT-ATCAGATGCACA	1.3 kb
418	13707-L15181	Exon 25	7007-7008	TGTGAAGGAGTT-CCTCATCTCCAG	22.8 kb
468	13708-L15182	Exon 26	7190-7191	TGCCCTGAGGAT-GGAGGTTCTGGG	1.5 kb
229	05641-L05091	Exon 26	8714-8715	TTTGGAACTAT-AACATAGCTGTC	
		<i>stop codon</i>	7225-7227 (Exon 26)		

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.

<< 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.

Related SALSA MLPA probemixes

- P011/P012 VWF Probes for the *VWF* gene involved in von Willebrand disease.
P207 F9 Probes for the *F7* and *F9* genes involved in factor VII deficiency and haemophilia B.
P440 F10 + F11 Probes for the *F10* and *F11* genes involved in factor X and factor XI deficiency.
P469 F5 Probes for the *F5* gene involved in factor V deficiency.

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Selected publications using SALSA MLPA Probemix P178 F8

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P178 Product history	
<i>Version</i>	<i>Modification</i>
B4	One reference probe has been replaced.
B3	Two reference probes have been removed.
B2	The 88 and 96 nt control fragments have been replaced (QDX2).
B1	Two F8 and five reference probes have been replaced, two reference probes have been added, one GDI flanking probe has been removed and several probes have been changed in length.
A1	First release.

Implemented changes in the product description

Version B4-01 – 13 December 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).
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- For uniformity, the chromosomal locations and bands in this document are now all based on hg18 (NCBI36).
- Probe remark regarding SNPs affecting the 247 nt probe 05617-L05067 and the 418 nt probe 13707-L15181 removed.
- SALSA MLPA probemix P469 F5 added to the related products.
- List of publications using SALSA MLPA probemix P178 F8 updated.

Version 14 – 04 February 2016 (55)

- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new pictures included).
- F8 exon numbering adjusted.
- Manufacturer's address adjusted.

Version 13 – 15 July 2015 (54)

- Figures based on the use of old MLPA buffer (replaced in December 2012) removed.

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

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