Product Description SALSA® MLPA® Probemix P184-C4 JAG1

To be used with the MLPA General Protocol.

Version C4. As compared to version C3, four reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P184-025R:** SALSA MLPA Probemix P184 JAG1, 25 reactions.
- **P184-050R:** SALSA MLPA Probemix P184 JAG1, 50 reactions.
- **P184-100R:** SALSA MLPA Probemix P184 JAG1, 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 P184 JAG1 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *JAG1* gene, which is associated with Alagille syndrome (ALGS).

ALGS causes a wide spectrum of clinical manifestations. Features are characteristic faces, with deep-set eyes and prominent forehead and chin. Cardiac defects are seen in the majority of ALGS patients. ALGS is also one of the major forms of chronic liver disease in childhood. Other abnormalities are seen in the eyes, vertebrae and kidneys. Defects in the *JAG1* gene on chromosome 20 are the main cause (94%) of ALGS. The disorder is inherited in an autosomal dominant manner.

The *JAG1* gene (26 exons) spans ~36 kb of genomic DNA and is located on 20p12.2, 11 Mb from the p-telomere.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1273/.

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 *JAG1* exon numbering used in this P184-C4 JAG1 product description is the exon numbering from the RefSeq transcript NM_000214.3, which is identical to the LRG_1191 sequence. The exon numbering and NM_ sequence used have been retrieved on 10/2020. 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 P184-C4 JAG1 contains 39 MLPA probes with amplification products between 136 and 454 nucleotides (nt). This includes 28 probes for the *JAG1* gene, one probe for each exon of the gene and two probes for exon 1 and 26. In addition, 11 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 Alagille syndrome. 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 numbers NA00981 and NA10608 from the Coriell Institute have been tested with this P184-C4 probemix at MRC-Holland and can be used as positive control samples to detect a heterozygous duplication and a heterozygous deletion of *JAG1*, respectively. 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 *JAG1* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P184 JAG1.
- 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.

***JAG1* mutation database:** https://databases.lovd.nl/shared/genes/JAG1. We strongly encourage users to deposit positive results in the Leiden Open Variation 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 *JAG1* exons 5 and 7 but not exon 6) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P184-C4 JAG1

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*JAG1*
64-105	Control fragments – see table in probemix content section for more information		
136 *	Reference probe 19976-L27038	4p16	
142	**JAG1** probe 14496-L16237		**Exon 1**
148	**JAG1** probe 05984-L10039		**Exon 13**
154	Reference probe 03857-L03308	17q11	
159	**JAG1** probe 05975-L05400		**Exon 2**
166	**JAG1** probe 05985-L05410		**Exon 14**
172	Reference probe 10071-L10495	8q22	
178	**JAG1** probe 05976-L05401		**Exon 3**
184	**JAG1** probe 05993-L05418		**Exon 26**
193	**JAG1** probe 05986-L05411		**Exon 15**
202	**JAG1** probe 02213-L01217		**Exon 22**
214	**JAG1** probe 05977-L16434		**Exon 4**
221	**JAG1** probe 05987-L05412		**Exon 17**
227 *	Reference probe 22485-L09033	11p15	
232	**JAG1** probe 14498-L16433		**Exon 11**
238	**JAG1** probe 05978-L05403		**Exon 5**
247	**JAG1** probe 05988-L05413		**Exon 20**
256	Reference probe 02469-L01913	15q21	
265	**JAG1** probe 05979-L05404		**Exon 6**
274	**JAG1** probe 05989-L05414		**Exon 21**
283	Reference probe 03686-L03101	14q22	
292	**JAG1** probe 05980-L05405		**Exon 8**
301	**JAG1** probe 14497-L16238		**Exon 1**
310	Reference probe 03603-L02970	1p31	
319	**JAG1** probe 05981-L05406		**Exon 9**
328	**JAG1** probe 05990-L10040		**Exon 23**
337 *	Reference probe 22436-L30035	22q11	
346	**JAG1** probe 05982-L05407		**Exon 10**
355	**JAG1** probe 05991-L05416		**Exon 24**
374	**JAG1** probe 05983-L05408		**Exon 12**
382	**JAG1** probe 05992-L05417		**Exon 26**
393	Reference probe 04838-L04222	5p13	
400	**JAG1** probe 09610-L09905		**Exon 7**
409	**JAG1** probe 09615-L09910		**Exon 25**
418	**JAG1** probe 21261-L29869		**Exon 18**
427 *	Reference probe 21831-L31602	16q21	
436	**JAG1** probe 09614-L09909		**Exon 19**
445	**JAG1** probe 09612-L09907		**Exon 16**
454	Reference probe 16407-L18832	3p22	

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

* New in version C4.

Table 2. *JAG1* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*JAG1* exon[a]	Ligation site NM_000214.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*470-472 (Exon 1)*		
142	14496-L16237	Exon 1	200-201	GAAGGAAAGAAA-GCCGGGAGGTGG	0.1 kb
301	14497-L16238	Exon 1	345-346	GCATGCTCCAAT-CGGCGGAGTATA	0.8 kb
159	05975-L05400	Exon 2	697-698	ACATACTTCAAA-GTGTGCCTCAAG	8.9 kb
178	05976-L05401	Exon 3	869-870	GGTCCTATACGT-TGCTTGTGGAGG	5.4 kb
214	05977-L16434	Exon 4	1028-1029	TCCGCGTGACCT-GTGATGACTACT	2.2 kb
238	05978-L05403	Exon 5	1201-1202	AAGCATGGGTCT-TGCAAACTCCCA	3.9 kb
265	05979-L05404	Exon 6	1258-1259	CTGTACTGTGAT-AAGTGCATCCCA	0.3 kb
400	09610-L09905	Exon 7	1364-1365	CAGATCTCAATT-ACTGTGGGACTC	0.6 kb
292	05980-L05405	Exon 8	1511-1512	CCTGTCACAACA-GAGGCAGCTGTA	1.4 kb
319	05981-L05406	Exon 9	1656-1657	GGTTAACGGATT-TAAGTGTGTGTG	0.7 kb
346	05982-L05407	Exon 10	1753-1754	AAATCCTGTAAG-AATCTCATTGCC	0.5 kb
232	14498-L16433	Exon 11	1848-1847 *reverse*	AGGAGGCGTCAT-TCTGACACTGGC	0.4 kb
374	05983-L05408	Exon 12	1937-1938	GAGACATCGATG-AATGTGCCAGCA	0.6 kb
148	05984-L10039	Exon 13	2105-2106	ACCGTGCCAGTG-ACTATTTCTGCA	1.1 kb
166	05985-L05410	Exon 14	2306-2307	CGGGAGGCAAAT-TCACCTGTGACT	1.0 kb
193	05986-L05411	Exon 15	2407-2408	ACTTGCATCGAT-GGTGTCAACTCC	0.6 kb
445	09612-L09907	Exon 16	2549-2550	ACTGTGACTGTA-AAAATGGGTGGA	0.2 kb
221	05987-L05412	Exon 17	2632-2633	GGCACCTGCTAT-GATGAGGGGGAT	0.3 kb
418	21261-L29869	Exon 18	2766-2765 *reverse*	AGACGCACGTAA-AGGACTCGCCGT	0.5 kb
436	09614-L09909	Exon 19	2823-2822 *reverse*	GAGGGCTGCAGT-CATTGGTATCTG	0.5 kb
247	05988-L05413	Exon 20	26 nt before exon 20	TGGGAGGTTGGT-AACCAAGGTGGT	1.3 kb
274	05989-L05414	Exon 21	2974-2975	GGAGCGACCTGT-GTGGATGAGATC	0.8 kb
202	02213-L01217	Exon 22	9 nt after exon 22	AAGGTAGGACAT-GATGGCTGCCGC	0.3 kb
328	05990-L10040	Exon 23	3328-3329	TCTGACTCCTAT-TACCAGGATAAC	0.3 kb
355	05991-L05416	Exon 24	3430-3431	AGGAATTTGAAT-ATTTTGAAGAAT	0.4 kb
409	09615-L09910	Exon 25	3615-3616	CAGCTCGCTGAT-TGCTGCCGTTGC	1.2 kb
382	05992-L05417	Exon 26	3951-3952	AGAGGACGACAT-GGACAAACACCA	1.8 kb
184	05993-L05418	Exon 26	5789-5790	ACTGAAAGGCTT-TTCAACCACAAA	
		stop codon	*4124-4126 (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.

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 P184 JAG1

- Cho JM et al. (2015). Clinical features, outcomes, and genetic analysis in Korean children with Alagille syndrome. *Pediatr Int*. 57:552-557.
- Dědič T et al. (2015). Alagille syndrome mimicking biliary atresia in early infancy. *PLoS One*. 10:e0143939.
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- Jurkiewicz D et al. (2014). Spectrum of JAG1 gene mutations in Polish patients with Alagille syndrome. *J Appl Genet*. 55:329-336.
- Kamath BM et al. (2009). SNP array mapping of chromosome 20p deletions: genotypes, phenotypes, and copy number variation. *Hum Mutat*. 30:371-378.

- Li L et al. (2015). JAG1 mutation spectrum and origin in Chinese children with clinical features of Alagille syndrome. *PloS One*. 10:e0130355.
- Nicastro E et al. (2019). Diagnostic yield of an algorithm for neonatal and infantile cholestasis integrating next-generation sequencing. *J pediatr*. 211:54-62.
- Ohashi K et al. (2017). Combined genetic analyses can achieve efficient diagnostic yields for subjects with Alagille syndrome and incomplete Alagille syndrome. *Acta Paediatr*. 106:1817-1824.
- Rajagopalan R et al. (2020). Genome sequencing increases diagnostic yield in clinically diagnosed Alagille syndrome patients with previously negative test results. *Genet Med*. 1-8.
- Togawa T et al. (2016). Molecular genetic dissection and neonatal/infantile intrahepatic cholestasis using targeted next-generation sequencing. *J pediatr*. 171:171-177.

P184 Product history	
Version	*Modification*
C4	Four reference probes have been replaced.
C3	One reference probe has been removed and two reference probes replaced and one probe length has been adjusted.
C2	Three reference probes have been replaced and the control fragments have been adjusted (QDX2).
C1	*JAG1* exon 1 probe has been replaced by two new exon 1 probes, one exon 11 probe and two new reference probes have been added.
B	Five additional *JAG1* probes have been added, two reference probes have been added and the control fragments have been adjusted.
A	First release.

Implemented changes in the product description

Version C4-01 — 19 November 2020 (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).
- Ligation sites of the probes targeting the *JAG1* gene updated according to new version of the NM_ reference sequence.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 12 – 28 April 2017 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- New references added on page 2.
- Various minor textual changes.

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