# Product Description
# SALSA® MLPA® Probemix P191-B4 X-linked Alport syndrome mix 1 & P192-B4 X-linked Alport syndrome mix 2

To be used with the MLPA General Protocol.

**Version B4**
For complete product history see page 9.

**Catalogue numbers:**
- **P191-025R:** SALSA MLPA Probemix P191 X-linked Alport syndrome mix 1, 25 reactions.
- **P191-050R:** SALSA MLPA Probemix P191 X-linked Alport syndrome mix 1, 50 reactions.
- **P191-100R:** SALSA MLPA Probemix P191 X-linked Alport syndrome mix 1, 100 reactions.

- **P192-025R:** SALSA MLPA Probemix P192 X-linked Alport syndrome mix 2, 25 reactions.
- **P192-050R:** SALSA MLPA Probemix P192 X-linked Alport syndrome mix 2, 50 reactions.
- **P192-100R:** SALSA MLPA Probemix P192 X-linked Alport syndrome mix 2, 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 Probemixes P191 X-linked Alport syndrome mix 1 and P192 X-linked Alport syndrome mix 2 are **research use only (RUO)** assays for the detection of deletions or duplications in the *COL4A5* gene, which is associated with X-linked Alport syndrome (XLAS).

Alport syndrome (AS) is an inherited disorder of the basement membrane, resulting in progressive renal failure due to glomerulonephropathy, variable sensorineural hearing loss, and variable ocular anomalies. It is a genetically heterogeneous disorder, with all forms resulting from mutations in the genes encoding the alpha-3 (COL4A3; 2q36.3), alpha-4 (COL4A4; 2q36.3), and alpha-5 (COL4A5; Xq22.3) chains of type IV collagen, which is a major structural component of the basement membrane (Nozu et al. 2019). AS can be divided into X-Linked AS (XLAS), autosomal recessive AS (ARAS), and autosomal dominant AS (ADAS). XLAS is caused by mutations in the COL4A5 gene and accounts for approximately 80% of AS, whereas ARAS and ADAS are caused by mutations in the COL4A3 and COL4A4 genes and account for approximately 15% and 5% of AS, respectively (Nozu K et al. 2019).

The first exon of *COL4A5* is located very close to the first exon of the *COL4A6* gene which encodes one of the other type IV collagen subunits. The two genes are arranged head-to-head and share a common promoter region. Deletions in the *COL4A5* gene that extend into the *COL4A6* gene result in Alport syndrome-diffuse leiomyomatosis (AS-DL)(Nozu K et al. 2017, Zhou X et al 2021).

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1207/

**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
Matched Annotation from NCBI and EMBL-EBI (MANE): http://www.ncbi.nlm.nih.gov/refseq/MANE/
Tark – Transcript Archive (MANE) database: http://tark.ensembl.org/
Locus Reference Genomic (LRG) database: http://www.lrg-sequence.org/

**Exon numbering**
The *COL4A5* exon numbering used in this P191-B4 X-linked Alport syndrome mix 1 and P192-B4 X-linked Alport syndrome mix 2 product description is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcript NM_033380.3, as indicated in Table 2. The *COL4A6* exon numbering used is the exon numbering from the MANE project (release version 1.0) based on MANE Select transcript NM_033641.4, as indicated in Table 2. Compared to the MANE select for *COL4A6*, NM_001847.4 utilizes an alternative exon 1 (exon 1A). 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 P191-B4 X-linked Alport syndrome mix 1 contains 37 MLPA probes with amplification products between 130 and 490 nucleotides (nt). This includes 26 probes for the *COL4A5* gene and one probe for the *COL4A6* gene. In addition, ten reference probes are included that detect locations on the X-chromosome. The SALSA MLPA Probemix P192-B4 X-linked Alport syndrome mix 2 contains 37 MLPA probes with amplification products between 130 and 444 nt. This includes 25 probes for the *COL4A5* gene and two probes for the *COL4A6* gene. 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.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 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 different unrelated individuals who are from families without a history of Alport syndrome. It is recommended to use samples of the same sex to facilitate interpretation. 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.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.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:

| Copy Number status: Male samples | Final ratio |
|---|---|
| Normal | 0.80 < FR < 1.20 |
| Deletion | FR = 0 |
| Duplication | 1.65 < FR < 2.25 |
| Ambiguous copy number | All other values |

| Copy Number status: Female samples | Final ratio |
|---|---|
| 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. 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 *COL4A5* gene are small (point) mutations, which will not be detected by using SALSA MLPA Probemix P191 X-linked Alport syndrome mix 1 and P192 X-linked Alport syndrome mix 2.
- 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.

**Alport mutation database:**
https://www.ncbi.nlm.nih.gov/clinvar/?term=col4a5%5Bgene%5D&redir=gene
https://databases.lovd.nl/shared/genes/COL4A5. We strongly encourage users to deposit positive results in the ClinVar database and/or 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 SNVs and unusual results (e.g., a duplication of *COL4A5* exons 4 and 6 but not exon 5) to MRC Holland: info@mrcholland.com.

## Table 1a. SALSA MLPA Probemix P191-B4 X-linked Alport syndrome mix 1

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *COL4A5* | *COL4A6* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 130 | Reference probe 22528-L31699 | Xq23 | | |
| 136 | **COL4A5 probe** 05851-L05251 | | **Exon 1** | |
| 142 | **COL4A5 probe** 05861-L08942 | | **Exon 9** | |
| 150 | **COL4A5 probe** 05871-L31708 | | **Exon 19** | |
| 155 | Reference probe 22533-L31884 | Xq21 | | |
| 160 ± | **COL4A5 probe** 05862-L21842 | | **Exon 10** | |
| 171 | **COL4A5 probe** 20677-L28487 | | **Exon 49** | |
| 185 | **COL4A5 probe** 20678-L28490 | | **Exon 2** | |
| 192 | **COL4A5 probe** 07781-L28674 | | **Exon 29** | |
| 197 | **COL4A5 probe** 05873-L28675 | | **Exon 21** | |
| 204 | **COL4A5 probe** 06774-L28676 | | **Exon 47** | |
| 213 | Reference probe 06039-L31709 | Xq13 | | |
| 220 | **COL4A5 probe** 05864-L05264 | | **Exon 12** | |
| 234 | **COL4A5 probe** 20679-L28728 | | **Exon 22** | |
| 245 | Reference probe 03646-L28729 | Xp22 | | |
| 256 | **COL4A5 probe** 22534-L31710 | | **Exon 3** | |
| 265 | **COL4A5 probe** 07782-L08950 | | **Exon 32** | |
| 270 | **COL4A5 probe** 05875-L08951 | | **Exon 23** | |
| 276 | Reference probe 02900-L26167 | Xq26 | | |
| 283 | **COL4A5 probe** 05893-L31711 | | **Exon 41** | |
| 292 | **COL4A5 probe** 05856-L31712 | | **Exon 4** | |
| 298 | **COL4A5 probe** 07783-L08953 | | **Exon 33** | |
| 314 | **COL4A5 probe** 05894-L31713 | | **Exon 44** | |
| 325 | Reference probe 07622-L31714 | Xp22 | | |
| 332 | **COL4A5 probe** 05867-L31715 | | **Exon 15** | |
| 341 | Reference probe 06882-L31716 | Xp22 | | |
| 358 | **COL4A5 probe** 18087-L31717 | | **Exon 45** | |
| 368 | **COL4A5 probe** 05858-L23781 | | **Exon 6** | |
| 385 | **COL4A5 probe** 05896-L31718 | | **Exon 46** | |
| 391 | Reference probe 03520-L02313 | Xp11 | | |
| 399 | **COL4A5 probe** 05859-L05259 | | **Exon 7** | |
| 420 | **COL4A5 probe** 05888-L23100 | | **Exon 36** | |
| 427 | **COL4A6 probe** 05906-L23101 | | | **Exon 2** |
| 444 | Reference probe 05613-L14948 | Xq28 | | |
| 463 | **COL4A5 probe** 07779-L07515 | | **Exon 26** | |
| 476 | **COL4A5 probe** 07778-L23669 | | **Exon 13** | |
| 490 | Reference probe 01433-L25440 | Xp21 | | |

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

± SNP rs145970300 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

## Table 1b. SALSA MLPA Probemix P192-B4 X-linked Alport syndrome mix 2

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | |
|---|---|---|---|---|
| | | Reference | *COL4A5* | *COL4A6* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | |
| 130 | Reference probe 22528-L31699 | Xq23 | | |
| 142 | **COL4A5 probe** 07780-L23675 | | **Exon 27** | |
| 148 | **COL4A5 probe** 05880-L23676 | | **Exon 28** | |
| 154 | **COL4A5 probe** 05889-L23677 | | **Exon 37** | |
| 164 | **COL4A5 probe** 05852-L31700 | | **Exon 1** | |
| 169 | **COL4A5 probe** 05872-L31701 | | **Exon 20** | |
| 179 | Reference probe 16678-L19251 | Xp11 | | |
| 184 | **COL4A5** probe 22529-L31702 | | **Exon 11** | |
| 190 | **COL4A5** probe 05882-L08948 | | **Exon 30** | |
| 196 | **COL4A5 probe** 20680-L28732 | | **Exon 50** | |
| 205 | **COL4A5 probe** 05891-L31703 | | **Exon 39** | |
| 211 | Reference probe 05620-L05070 | Xq28 | | |
| 220 | **COL4A5 probe** 05854-L05254 | | **Exon 2** | |
| 233 | **COL4A5 probe** 05883-L23678 | | **Exon 31** | |
| 240 | **COL4A5 probe** 05901-L28731 | | **Exon 51** | |
| 247 | Reference probe 07105-L06685 | Xp22 | | |
| 260 | **COL4A5 probe** 06775-L31704 | | **Exon 48** | |
| 267 | **COL4A5 probe** 22530-L28730 | | **Exon 13** | |
| 274 | **COL4A5 probe** 05902-L05302 | | **Exon 52** | |
| 283 | Reference probe 04996-L04382 | Xq26 | | |
| 293 | **COL4A5 probe** 05866-L05266 | | **Exon 14** | |
| 304 | **COL4A5 probe** 05876-L31705 | | **Exon 24** | |
| 311 | **COL4A5 probe** 05903-L05303 | | **Exon 53** | |
| 317 | Reference probe 03045-L01042 | Xp21 | | |
| 328 | **COL4A5 probe** 05857-L05257 | | **Exon 5** | |
| 341 | **COL4A5 probe** 05886-L31706 | | **Exon 34** | |
| 350 | **COL4A6 probe** 05904-L31707 | | | **Exon 1A** |
| 358 | Reference probe 13748-L31885 | Xp22 | | |
| 364 | **COL4A5 probe** 20682-L28494 | | **Exon 35** | |
| 373 | **COL4A5 probe** 05868-L12806 | | **Exon 16** | |
| 381 | **COL4A6 probe** 21739-L05305 | | | **Exon 1** |
| 391 | Reference probe 03520-L02313 | Xp11 | | |
| 406 | **COL4A5 probe** 20683-L28495 | | **Exon 17** | |
| 418 | Reference probe 06187-L02096 | Xq13 | | |
| 427 | **COL4A5 probe** 07784-L07520 | | **Exon 38** | |
| 436 | **COL4A5 probe** 05870-L08957 | | **Exon 18** | |
| 444 | Reference probe 22532-L22564 | Xq25 | | |

## Table 2. P191/P192 probes arranged according to chromosomal location

| Length (nt) P191 | Length (nt) P192 | SALSA MLPA probe | exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|---|
| | | | ***COL4A6*** | **NM_033641.4** | | |
| | | | *stop codon* | *5297-5299* | | |
| 427 | | 05906-L23101 | Exon 2 | 242-243 | ATTTCAGGTTGT-GGCTGCTCCTGG | 0.4 kb |
| | | | *start codon* | *227-229* | | |
| | 381 | 21739-L05305 | Exon 1 | 21-22 | GTGCTGCAAGTT-GTAACGGGCACC | 1.1 kb |
| | 350 | 05904-L31707 | 1A | NM_001847.4; 2 nt before alternative exon 1A | CCCTGGGCTGCT-GGTCTTCTTTAC | 0.6 kb |
| | | | | | | |
| | | | ***COL4A5*** | **NM_033380.3** | | |
| 136 | | 05851-L05251 | Exon 1 | 167-168 | CTCTTCACCCAA-GCCTCACTGTCC | 0.2 kb |
| | | | *start codon* | *289-291* | | |
| | 164 # | 05852-L31700 | Exon 1 | 329-330 | CGGCTTGTTCTT-ACTGGCCCTGAG | 99.5 kb |
| 185 | | 20678-L28490 | Exon 2 | Intron 1, 63 nt before exon 2 | AGTTGAGCTGTA-AGTCAGAGTCTG | 0.1 kb |
| | 220 | 05854-L05254 | Exon 2 | 411-412 | TGTGACTGCAGT-GGCATAAAAGGG | 19.3 kb |
| 256 | | 22534-L31710 | Exon 3 | 456-457 | CCAGGTTTGGAA-GGACACCCAGGA | 4.8 kb |
| 292 | | 05856-L31712 | Exon 4 | 536-537 | TGATGGAATTCC-AGGGCCACCAGG | 4.8 kb |
| | 328 | 05857-L05257 | Exon 5 | 586-587 | GACTTCCTGGAT-TTCCAGGGACAC | 0.1 kb |
| 368 | | 05858-L23781 | Exon 6 | 636-637 | GATGGGGCCCCA-GGACCTCAAGGT | 2.6 kb |
| 399 | | 05859-L05259 | Exon 7 | 690-691 | CGTGGATTTCCA-GGCAGTCCCGGT | 2.1 kb |
| | | *No probe* | Exon 8 | | | |
| 142 | | 05861-L08942 | Exon 9 | 758-759 | CTTTTAGGGTGA-ACCAGGTAGTAT | 2.4 kb |
| 160 ± | | 05862-L21842 | Exon 10 | 861-862 | ACTGGTATACCA-GGGCCAATTGGT | 2.0 kb |
| | 184 | 22529-L31702 | Exon 11 | 906-907 | TAGGGCCCTCCT-GGTCCACCAGGA | 0.1 kb |
| 220 | | 05864-L05264 | Exon 12 | 942-943 | CAGGGGAATATG-GGCTTAAATTTC | 0.2 kb |
| | 267 | 22530-L28730 | Exon 13 | 1002-1003 | CAGGGCCCACCT-GGGCCACCTGGG | 0.03 kb |
| 476 | | 07778-L23669 | Exon 13 | 1039-1040 | AGAAAAGACCAA-TTGATGTAGAGT | 2.2 kb |
| | 293 | 05866-L05266 | Exon 14 | 1080-1081 | GGACTTCCTGGT-GACCGAGGGCCT | 0.2 kb |
| 332 | | 05867-L31715 | Exon 15 | 1137-1138 | CCCCCAGGTGGT-GAGAAAGGTGAG | 0.3 kb |
| | 373 | 05868-L12806 | Exon 16 | 1207-1208 | ATGGAGAAAATG-GCCAACCAGGAA | 1.9 kb |
| | 406 | 20683-L28495 | Exon 17 | 1228-1229 | TTTTAAAGGGTT-TGCCTGGTGATC | 1.6 kb |
| | 436 | 05870-L08957 | Exon 18 | 1296-1297 | AAAGGTGACACT-GGCCCACCTGGA | 2.2 kb |
| 150 | | 05871-L31708 | Exon 19 | 1411-1412 | GAGAGCGAGGAT-TTCCTGGAATAC | 4.4 kb |
| | 169 | 05872-L31701 | Exon 20 | 1489-1490 | GCCCTCCTGGAT-TTCCTGGAGAAA | 0.5 kb |
| 197 | | 05873-L28675 | Exon 21 | 1656-1657 | CCAGGCCCTCCA-GGCCCCCCAGGA | 3.9 kb |
| 234 | | 20679-L28728 | Exon 22 | 1738-1739 | CTTGCTTCAACT-GCATTGGAACTG | 1.5 kb |
| 270 | | 05875-L08951 | Exon 23 | 1830-1831 | CCTGGACAGAAA-GGGGAAAAAGGA | 0.5 kb |
| | 304 | 05876-L31705 | Exon 24 | 2013-2014 | CCTGGTTTACCT-GGCACTCCTGGA | 3.9 kb |
| | | *No probe* | Exon 25 | | | |
| 463 | | 07779-L07515 | Exon 26 | 2263-2264 | CAGGTCAGACTA-TAACCCAGCCGG | 0.6 kb |
| | 142 | 07780-L23675 | Exon 27 | 2421-2422 | CTTCCTGGACCA-CCTGGTCCCAAA | 1.0 kb |
| | 148 | 05880-L23676 | Exon 28 | 2463-2464 | GGACCTCCAGGA-GCACCTGGGACA | 3.9 kb |
| 192 | | 07781-L28674 | Exon 29 | 2661-2662 | CGCACTGGCTTA-GATGGGCTCCCT | 8.1 kb |
| | 190 | 05882-L08948 | Exon 30 | 2709-2710 | AATGGACAACCT-GGACCAATGGGA | 5.4 kb |
| | 233 | 05883-L23678 | Exon 31 | 2832-2833 | AAGGGGGATCCA-GGACCTCCTGGA | 1.6 kb |
| 265 | | 07782-L08950 | Exon 32 | 3051-3052 | GTACCTGGTCTT-AAAGGTAATAAT | 0.8 kb |
| 298 | | 07783-L08953 | Exon 33 | 3086-3087 | TCAGCCAGGACT-TCCTGGCCCTAC | 1.6 kb |
| | 341 | 05886-L31706 | Exon 34 | 3235-3236 | GGCCAAAAGGTT-ATCAGGGTTTGC | 1.5 kb |
| | 364 | 20682-L28494 | Exon 35 | 3330-3331 | CCTGGTCTCCCT-GGACAGCCAGGT | 0.6 kb |
| 420 | | 05888-L23100 | Exon 36 | 3486-3487 | GGCGACAAAGGT-GATCCTGGTATT | 29.1 kb |
| | 154 | 05889-L23677 | Exon 37 | 3561-3562 | CCTGGATACCCA-GGGAACCCTGGT | 10.2 kb |

| Length (nt) P191 | Length (nt) P192 | SALSA MLPA probe | exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|---|
| | 427 | 07784-L07520 | Exon 38 | 3726-3725 reverse | GGACCAGGTTCT-CCTGGAAGGCCG | 1.0 kb |
| | 205 | 05891-L31703 | Exon 39 | 3763-3764 | GTCATCCTGGGC-AACCAGGGCCTC | 2.0 kb |
| | | *No probe* | *Exon 40* | | | |
| 283 | | 05893-L31711 | Exon 41 | 4038-4039 | CTGGAAGGACCT-AAAGGCAACCCT | 9.1 kb |
| | | *No probe* | | | | |
| | | *No probe* | | | | |
| 314 | | 05894-L31713 | Exon 44 | 4119-4120 | CCAGAAGGTCCT-CCAGGTCTCCCT | 3.2 kb |
| 358 | | 18087-L31717 | Exon 45 | 4268-4269 | TGGAATGAAAGG-AGATCCTGGTCT | 0.2 kb |
| 385 | | 05896-L31718 | Exon 46 | 4329-4330 | AGTGGAGTACCT-GGATCAGCTGGC | 0.9 kb |
| 204 | | 06774-L28676 | Exon 47 | 4453-4454 | GAATCCCTGGCC-AGCCTGGGCTAA | 4.2 kb |
| | 260 | 06775-L31704 | Exon 48 | 4530-4531 | CCAGGAGATCCT-GGACGCAATGGA | 1.6 kb |
| 171 | | 20677-L28487 | Exon 49 | 4732-4733 | ATGCACCACAAT-GCCCACAGGGAA | 5.3 kb |
| | 196 | 20680-L28732 | Exon 50 | 4952-4953 | GCCAATGAGCAT-GCAACCCCTAAA | 2.0 kb |
| | 240 | 05901-L28731 | Exon 51 | 5025-5026 | CCAGCTGTGGTG-ATCGCAGTTCAC | 0.6 kb |
| | 274 | 05902-L05302 | Exon 52 | 5239-5240 | ATGCCAACTCCT-ACAGCTTTTGGC | 1.9 kb |
| | | | *stop codon* | *5362-5364* | | |
| | 311 | 05903-L05303 | Exon 53 | 6321-6322 | GAATCCTCCTGT-GGCCTCTGCTTG | |

[a] See section Exon numbering on page 2 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.

# This probe's specificity relies on a single nucleotide difference compared to a similar sequence located in the same chromosome. As a result, an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related sequence in chromosome X.
± SNP rs145970300 could influence the probe signal. In case of apparent deletions, it is recommended to sequence the region targeted by this probe.

SNVs located in the target sequence of a probe can influence probe hybridization and/or probe ligation. Please note: not all known SNVs are mentioned in the tables above. Single probe aberration(s) must be confirmed by another method.

## Related SALSA MLPA probemixes

| | |
|---|---|
| P439 COL4A3 | Contains probes for the *COL4A3* gene involved in ARAS and ADAS. |
| P444 COL4A4 | Contains probes for the *COL4A4* gene involved in ARAS and ADAS. |

## References


- Nozu K et al. (2017). Characterization of contiguous gene deletions in COL4A6 and COL4A5 in Alport syndrome-diffuse leiomyomatosis. J Hum Genet. Jul;62(7):733-735.
- Nozu K et al. (2019). A review of clinical characteristics and genetic backgrounds in Alport syndrome. Clin Exp Nephrol. 23:158–168.
- 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.
- Zhou X et al. (2021). Clinical Manifestations of Alport Syndrome-Diffuse Leiomyomatosis Patients With Contiguous Gene Deletions in *COL4A6* and *COL4A5*. Front Med (Lausanne). 27;8:766224.

## Selected publications using SALSA MLPA Probemix P191 X-linked Alport syndrome mix 1 & P192 X-linked Alport syndrome mix 2

▪ Aoto Y et al. (2020). A case with somatic and germline mosaicism in COL4A5 detected by multiplex ligation-dependent probe amplification in X-linked Alport syndrome. CEN Case Rep; 9:431–436.
▪ Hadjipanagi D et al. (2022). Novel and Founder Pathogenic Variants in X-Linked Alport Syndrome Families in Greece. Genes; 13(12):2203.
▪ Hertz JM et al. (2008). MLPA and cDNA improves COL4A5 mutation detection in X-linked Alport syndrome. Clin Genet. 74:522-30.
▪ Sá MJ (2013). Deletion of the 5'exons of COL4A6 is not needed for the development of diffuse leiomyomatosis in patients with Alport syndrome. J Med Genet. 50:745-53
▪ Uliana et al. (2011). Alport syndrome and leiomyomatosis: the first deletion extending beyond COL4A6 intron 2. Pediatr Nephrol. 26:717-24.
▪ Yamamura, T et al. (2019). Comparison between conventional and comprehensive sequencing approaches for genetic diagnosis of Alport syndrome. Mol Genet Genomic Med; 7:e883.

| **P191 product history** | |
|---|---|
| *Version* | *Modification* |
| B4 | As compared to version B3, twelve probes (eight target and four reference) had a minor alteration, no change in sequence detected and two reference probes were replaced by three new reference probes. |
| B3 | One reference probe has been replaced and one removed |
| B2 | Four reference probes have been replaced. |
| B1 | Several probes have been exchanged with P192 in order to minimize the generation of aspecific amplification products, especially in the no DNA control reactions. |

| **P192 product history** | |
|---|---|
| *Version* | *Modification* |
| B4 | As compared to version B3, twelve probes (ten target and two reference) had a minor alteration, no change in sequence detected and three reference probes were replaced. |
| B3 | One reference probe has been replaced, and the length of several probes adjusted. |
| B2 | Four reference probes have been replaced. |
| B1 | Several probes have been exchanged with P191 in order to minimize the generation of aspecific amplification products, especially in the no DNA control reactions. |

| **Implemented changes in the product description** |
|---|
| *Version B4-02 –19 May 2023 (04P)*<br>- Product name adjusted to X-linked Alport syndrome<br>- Product description rewritten and adapted to a new template.<br>- Warning added to Table 1a and 2. indicating that SNP rs145970300 could influence the COL4A5 probe (05862-L21842) signal.<br>- Warning added to Table 2. for probe specificity relying on a single nucleotide difference between target gene and related gene or pseudogene.<br>- Removed “no probe” indication in Table 2 for exon 44.<br>- Sections “Related SALSA MLPA probemixes” and “Selected publications” have been updated.<br>*Version B4-01 — 15 August 2019 (02P)*<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *COL4A5* and *COL4A6* genes updated according to new version of the NM_ reference sequence. |

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