# Product Description SALSA® MLPA® Probemix P160-C1 STS

To be used with the MLPA General Protocol.

**Version C1.** For complete product history see page 7.

**Catalogue numbers:**
- **P160-025R:** SALSA MLPA Probemix P160 STS, 25 reactions.
- **P160-050R:** SALSA MLPA Probemix P160 STS, 50 reactions.
- **P160-100R:** SALSA MLPA Probemix P160 STS, 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 P160 STS is a **research use only (RUO)** assay for the detection of deletions or duplications in the *STS* gene, which is associated with X-linked ichthyosis. This probemix can also be used to detect *STS* deletions or duplications that extend into the neighbouring *ANOS1* (*KAL1*) and *NLGN4X* genes.

X-linked ichthyosis is a skin disorder characterised by dark brown and black, polygonal scales particularly found on the trunk, neck and extremities, which are caused by a deficiency of the steroid sulfatase (STS) enzyme. Placental steroid sulfatase deficiency occurs in pregnant mothers of affected males and is associated with low oestrogen production, failed induction of and prolonged labour. In up to 90% of patients, X-linked ichthyosis is caused by complete deletion of the *STS* gene located on chromosome Xp22.31 (Hernández-Martín et al. 1999), but partial *STS* gene deletions and point mutations have also been described.

Larger deletions at Xp22.31 might include neighbouring genes such as *ANOS1* or *NLGN4X.* Loss of the *ANOS1* gene causes 5-10% of Kallmann syndrome, which is characterised by impaired sense of smell (anosmia/hyposmia) and delayed or absent puberty due to hypogonadotropic hypogonadism. Copy number variations and mutations in the *NLGN4X* gene have been associated with X-linked intellectual disability and autism.

**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 *STS*, *ANOS1* and *NLGN4X* exon numbering used in this P160-C1 STS product description is the exon numbering from the RefSeq records NG_021472.2, NG_007088.2 and NG_008881.2, respectively. For flanking probes located in the genes *PUDP* and *GPR143*, the RefSeq records NG_021256.1 and NG_009074.1 were used for exon numbering. The exon numbering has been retrieved on 02/2020. 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 P160-C1 STS contains 35 MLPA probes with amplification products between 130 and 427 nucleotides (nt). This includes 13 probes for the *STS* gene, one probe for each exon with the exception of exon 3, as well as two flanking probes targeting the *PUDP* gene. Furthermore, three probes for the *ANOS1* gene and one flanking probe for the *GPR143* gene, as well as five probes for the *NLGN4X* gene are included. In addition, 11 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 X-linked ichthyosis or other Xp22-related disorders. 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.

**Positive control DNA samples:** MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. The quality of cell lines can change; therefore samples should be validated before use.

**Data analysis:** Coffalyser.Net software should be used for data analysis in combination with the appropriate lot-specific MLPA Coffalyser sheet. For both, the latest version should be used. Coffalyser.Net software is freely downloadable at www.mlpa.com. Use of other non-proprietary software may lead to inconclusive or false results. For more details on MLPA quality control and data analysis, including normalisation, see the Coffalyser.Net Reference Manual.

**Interpretation of results:** The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the dosage quotient (DQ) of each individual reference probe in the patient samples

should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the DQ of the probes can be used to interpret MLPA results:

| **Copy number status: Male samples** | **Dosage quotient** |
|---|---|
| Normal | 0.80 < DQ < 1.20 |
| 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. 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 *ANOS1* and *NLGN4X* genes are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P160 STS.
- 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.

**LOVD mutation database:** https://databases.lovd.nl/shared/genes/. We strongly encourage users to deposit positive results in the Leiden Open Variation Database (LOVD). Recommendations for the nomenclature to describe deletions/duplications of one or more exons can be found on http://varnomen.hgvs.org/.

Please report copy number changes detected by the reference probes, false positive results due to SNPs and unusual results (e.g., a duplication of *STS* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

## Table 1. SALSA MLPA Probemix P160-C1 STS

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *STS* | *ANOS1* | *NLGN4X* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | |
| 130 | Reference probe 13497-L13693 | Xq12 | | | |
| 136 | **STS probe** 05184-L04565 | | **Exon 1** | | |
| 142 | **NLGN4X probe** 05589-L04580 | | | | **Exon 3** |
| 148 | **ANOS1 probe** 04431-L03817 | | | **Exon 14** | |
| 154 | Reference probe 01355-L01615 | Xp21 | | | |
| 160 | **STS probe** 21041-L29265 | | **Exon 1** | | |
| 166 | **ANOS1 probe** 04423-L05579 | | | **Exon 7** | |
| 172 | Reference probe 06797-L06389 | Xq27 | | | |
| 178 | **STS probe** 05185-L04566 | | **Exon 2** | | |
| 183 | **NLGN4X probe** 05197-L04578 | | | | **Intron 1** |
| 190 | **STS probe** 21006-L05112 | | **Exon 10** | | |
| 196 | Reference probe 10757-L11353 | Xq28 | | | |
| 201 | **STS probe** 05190-L04571 | | **Exon 7** | | |
| 209 | **ANOS1 probe** 04425-L05940 | | | **Exon 9** | |
| 220 ¬ | **GPR143 probe** 02975-L02406 | | | **Upstream** | |
| 226 | **STS probe** 21042-L29266 | | **Exon 7** | | |
| 238 ¬ « | **PUDP robe** 05194-L04575 | | **Upstream** | | |
| 247 | **STS probe** 21043-L29267 | | **Exon 6** | | |
| 263 | **STS probe** 05586-L05589 | | **Exon 10** | | |
| 283 | **NLGN4X probe** 05587-L29518 | | | | **Intron 1** |
| 301 | Reference probe 01279-L00963 | Xq22 | | | |
| 310 | **STS probe** 05191-L04572 | | **Exon 8** | | |
| 319 | **NLGN4X probe** 05202-L04583 | | | | **Exon 6** |
| 326 | **STS probe** 21045-L29269 | | **Exon 6** | | |
| 337 | Reference probe 05121-L04511 | Xq26 | | | |
| 346 | **STS probe** 05187-L04568 | | **Exon 4** | | |
| 355 | **NLGN4X probe** 05588-L04579 | | | | **Exon 2** |
| 364 | Reference probe 02641-L02108 | Xp11 | | | |
| 373 | Reference probe 06464-L05990 | Xp22 | | | |
| 382 | **STS probe** 05192-L04573 | | **Exon 9** | | |
| 391 ¬ | **PUDP probe** 21046-L29270 | | **Upstream** | | |
| 399 | Reference probe 07657-L07363 | Xp11 | | | |
| 409 | Reference probe 00820-L00338 | Xq26 | | | |
| 418 | **STS probe** 05188-L04569 | | **Exon 5** | | |
| 427 | Reference probe 12797-L13935 | Xq21 | | | |

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

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
« 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. Xp22 probes arranged according to chromosomal location

| Length (nt) | SALSA MLPA probe | Gene/ Exon[a] | Ligation site | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | ***NLGN4X*** | **NM_181332.3** | | |
| | | *stop codon* | *3081-3083 (Exon 6)* | | |
| 319 | 05202-L04583 | Exon 6 | 3218-3219 | CTGACTTAAGAC-AAAAATGCAAAA | 136.7 kb |
| 142 | 05589-L04580 | Exon 3 | 1136-1137 | AAGAAGCCCGTC-ATGGTCTATATC | 122.1 kb |
| 355 | 05588-L04579 | Exon 2 | 637-638 | TTGAACCATGTC-ACGGCCCCAGGG | 75.5 kb |
| 183 # | 05197-L04578 | Intron 1 | 1561 nt after exon 1 | AGAGAGAGAGTG-AACTTCAGTCCT | 0.8 kb |
| 283 ൭ | 05587-L29518 | Intron 1 | 754 nt after exon 1 | GACGGCTTGGGT-GATGCACGAAAT | 822.4 kb |
| | | *start codon* | *633-635 (Exon 2)* | | |
| | | | | | |
| 391 ¬ | 21046-L29270 | *PUDP* Exon 4 | | GATGGAAACTCC-AATCTCAGGAGG | 97.9 kb |
| 238 ¬ « | 05194-L04575 | *PUDP* Exon 1 | | AGGCGGCGGCTG-CACACTACCCAC | 71.5 kb |
| | | | | | |
| | | ***STS*** | **NM_000351.7** | | |
| | | *start codon* | *236-238 (Exon 2)* | | |
| 160 | 21041-L29265 | Exon 1 | 98-97 reverse | TATTCCCATGAG-AAACCTCTGCTT | 0.1 kb |
| 136 | 05184-L04565 | Exon 1 | 190-191 | CATCACAGCTCA-GTTCCCCAACAA | 33.6 kb |
| 178 | 05185-L04566 | Exon 2 | 286-285 reverse | CTTGATGCTGCG-TGGCTCTCGGCT | 4.3 kb |
| | No probe | Exon 3 | | | |
| 346 | 05187-L04568 | Exon 4 | 522-523 | CCGCACTGGAGT-TTTCCTCTTCAC | 2.1 kb |
| 418 | 05188-L04569 | Exon 5 | 851-852 | GGCTACTCCACG-TGCCTCTAGGCG | 16.3 kb |
| 326 | 21045-L29269 | Exon 6 | 78 nt before exon 6 reverse | CATTCTGATTAA-GATCCACCTATT | 0.2 kb |
| 247 | 21043-L29267 | Exon 6 | 6 nt after exon 6 reverse | CACTGAGGAGAA-ACGTACCCACAC | 28.9 kb |
| 226 | 21042-L29266 | Exon 7 | 96 nt before exon 7 | AACGTTTACTTC-CACCTTGAGAAA | 0.2 kb |
| 201 | 05190-L04571 | Exon 7 | 1264-1265 | GCACATGTAGAA-GAAGTGTCTTCC | 20.3 kb |
| 310 | 05191-L04572 | Exon 8 | 1428-1429 | CAACATGGACAT-ATTTCCTACAGT | 8.6 kb |
| 382 | 05192-L04573 | Exon 9 | 1527-1528 | AAAAAGCCAACG-CTCCGATCATGA | 17.4 kb |
| 190 | 21006-L05112 | Exon 10 | 3109-3110 | CATGATTTTGTT-TTCATCCCATTT | 0.4 kb |
| 263 | 05586-L05589 | Exon 10 | 3507-3508 | TGAAAGTTGGCT-ATAATTTCTCTA | 1177.9 kb |
| | | *stop codon* | *1970-1972 (Exon 10)* | | |
| | | | | | |
| | | ***ANOS1*** | **NM_000216.4** | | |
| | | *stop codon* | *2142-2144 (Exon 14)* | | |
| 148 | 04431-L03817 | Exon 14 | 5418-5419 | ATGATCTTTACT-GAATTTGCCCTT | 24.3 kb |
| 209 | 04425-L05940 | Exon 9 | 1385-1386 | CCCACTCGCCCG-CTGGAAGTCGGA | 16.5 kb |
| 166 | 04423-L05579 | Exon 7 | 1105-1104 reverse | TACTGCTGACCA-TCCAGCTCCAAA | 1155.2 kb |
| | | *start codon* | *102-104 (Exon 1)* | | |
| | | | | | |
| 220 ¬ | 02975-L02406 | *GPR143* Exon 9 | | CTGCAACAAAAA-TGAGGGTGACCC | |

**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.

¬ Flanking probe. Included to help determine the extent of a deletion/duplication. Copy number alterations of only the flanking or reference probes are unlikely to be related to the condition tested.
« 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.
൭ The target sequence of this probe is not present in NM_181332.3 (transcript variant 2), but can be found in NM_020742.3 (transcript variant 1; ligation site 61-62 in exon 1).
# The target sequence of this probe is not present in NM_181332.3 (transcript variant 2), but can be found in NM_001282146.1 (transcript variant 4; ligation site 224-225 in exon 1).

## Related SALSA MLPA probemixes

- P054 FOXL2-TWIST1 — Contains probes for the *GPR143* gene.
- P132 Kallmann-1 — Contains probes for the *ANOS1* gene, which is associated with Kallmann syndrome.
- P133 Kallmann-2 — Contains probes for the *FGFR1*, *GNRHR*, *KISSR1*, *GNRH1*, *NELF, PROK2* and *PROKR2* genes, which are associated with Kallmann syndrome.

## References


- Hernández-Martín A et al. (2001). X-linked ichthyosis: an update. *Br J Dermatol.* 141:617-627.
- 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 P160 STS


- Diociaiuti A et al. (2018). X-linked ichthyosis: Clinical and molecular findings in 35 Italian patients. *Exp Dermatol.* 28:1156-1163.
- Diociaiuti A et al. (2019). Next generation sequencing uncovers a rare case of X-linked ichthyosis in an adopted girl homozygous for a novel nonsense mutation in the STS gene. *Acta Derm Venereol.* 99:828-830.
- Idkowiak J et al. (2016). Steroid sulfatase deficiency and androgen activation before and after puberty. *J Clin Endocrinol Metab*. 101:2543-53.
- Karampetsou E et al. (2014). Confined placental mosaicism: implications for fetal chromosomal analysis using microarray comparative genomic hybridization. *Prenat Diagn.* 34:98-101.
- Winge MCG et al. (2011). Filaggrin genotype determines functional and molecular alterations in skin of patients with atopic dermatitis and ichthyosis vulgaris. *PLoS One.* 6:e28254.
- Winge MCG et al. (2011). Novel point mutation in the STS gene in a patient with X-linked recessive ichthyosis. *J Dermatol Sci.* 63:62-64.


| **P160 Product history** | |
|---|---|
| *Version* | *Modification* |
| C1 | Two STS probes have been added, and one flanking probe and the Y-fragment on 118 nt have been removed. Two STS probes, two flanking probes and one reference probe have been replaced. In addition, several probe lengths have been adjusted. |
| B1 | Y-fragment on 118 nt and five reference probes have been replaced, control fragments have been adjusted (QDX2). |
| A2 | One STS probe has a different length and two reference probes have been replaced. In addition, four extra control fragments have been included at 88-96-100-105 nt. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| *Version C1-02 — 29 November 2024 (02P)*<br>- The term 'mental retardation' is considered outdated and was updated to 'intellectual disability' where appropriate<br>*Version C1-01 — 20 March 2020 (02P)*<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *STS*, *ANOS1* and *NLGN4X* genes updated according to new version of the NM_ reference sequence. |

| **More information: www.mlpa.com; www.mlpa.eu** | |
|---|---|
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