# Product Description
# SALSA® MLPA® Probemix P242-C1 Pancreatitis

To be used with the MLPA General Protocol.

**Version C1**
For complete product history see page 8.

**Catalogue numbers:**
- **P242-025R:** SALSA MLPA Probemix P242 Pancreatitis, 25 reactions.
- **P242-050R:** SALSA MLPA Probemix P242 Pancreatitis, 50 reactions.
- **P242-100R:** SALSA MLPA Probemix P242 Pancreatitis, 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.

SALSA MLPA Probemix P242 Pancreatitis contains probes for the *PRSS1* gene. *PRSS1* is located in a genomic region known to be excised during T cell maturation. The apparent copy number for *PRSS1* in a sample will therefore be dependent on the percentage of DNA derived from mature T cells. As a result, the PRSS1 probes show more variability as compared to the other probes present in this probemix.

It is strongly recommended to use selected reference samples, these can be chosen by testing several samples from healthy individuals. These reference samples should be derived from individuals of a similar age to most test samples, from similar test material, and have an intermediate *PRSS1* copy number (ratio 1). **Even with selected reference samples, it is possible to obtain low or high PRSS1 probe ratios (0.8 or 1.2)** in certain samples due to an unusually high or low percentage of mature T cells, respectively.

Note: Sample types that do not have T cell rearrangements (e.g. cell lines) should not be used as references samples for P242.

**General information**
The SALSA MLPA Probemix P242 Pancreatitis is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PRSS1*, *SPINK1*, and *CTRC* genes, which are associated with Hereditary Pancreatitis.

Hereditary Pancreatitis (HP; OMIM # 167800) is characterised by recurrent episodes of inflammation of the pancreas that progresses to chronic pancreatitis. HP has an autosomal dominant pattern of inheritance, and is defined by two or more individuals with pancreatitis in two or more generations of a family, or pancreatitis associated with a known germline pathogenic variant.

*PRSS1* encodes trypsin-1 (cationic trypsinogen), a major pancreatic digestive serine peptidase enzyme, which is produced and secreted by the pancreas. *PRSS1* pathogenic variants typically result in a trypsin protein that is either prematurely activated, while it is still in the pancreas, or resistant to degradation. Duplication of *PRSS1*, leading to an increase of the protein, also causes HP. Furthermore, gene conversion between *PRSS1* and *PRSS2* has been reported (Nemeth and Sahin-Toth 2014, Rygiel et al. 2015). In 90% of the cases, hereditary

pancreatitis is caused by two point mutations in the *PRSS1* gene, R122H (~65%) and N29I (25%) (Nemeth and Sahin-Toth 2014). Gene conversion between *PRSS1* and *PRSS2* exon 2 and the subsequent intron can occur, resulting in the N29I mutation together with N54S, a non-pathogenic mutation (Teich et al. 2005). In less than 6% of the cases, HP is caused by *PRSS1* (large) deletions/duplications.

*SPINK1* encodes the serine protease inhibitor Kazel-type 1. Pathogenic variants in *SPINK1* can lead to autosomal recessive pancreatitis, and have been identified in approximately 20% of families with hereditary pancreatitis, without a *PRSS1* germline pathogenic variant. The risk for developing acute pancreatitis is highly increased when the N34S mutation in *SPINK1* is present (Koziel et al. 2015).

*CTRC* encodes chymotrypsin C (CTRC), a low-abundance pancreatic digestive enzyme that is synthetized with PRSS1. CTRC is important for the degradation of the prematurely activated trypsin within the pancreas. Pathogenic variants of *CTRC* have been associated with chronic pancreatitis (Masson et al. 2008).

While identification of a heterozygous *PRSS1* pathogenic variant confirms a diagnosis of hereditary pancreatitis, the presence of isolated pathogenic variants in the *SPINK1* or *CTRC* genes is insufficient to cause pancreatitis (Masson et al. 2013).

More information is available at: https://www.ncbi.nlm.nih.gov/books/NBK190101/; https://www.ncbi.nlm.nih.gov/books/NBK84399/.

**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 *PRSS1*, *SPINK1*, and *CTRC* exon numbering used in this P242-C1 Pancreatitis product description is the exon numbering from the LRG_1013, NM_003122.5, and NG_009253.2 sequences, respectively. The exon numbering of the NM_ sequence that was used for determining a probe's ligation site does not always correspond to the exon numbering obtained from the LRG/NG sequences. As changes to the databases can occur after release of this product description, the NM_ sequence and exon numbering may not be up-to-date.

**Probemix content**
The SALSA MLPA Probemix P242-C1 Pancreatitis contains 32 MLPA probes with amplification products between 136 and 436 nucleotides (nt). This includes six probes for the *PRSS1* gene, one probe for each exon and two probes for exon 1. Furthermore, it contains one upstream and two downstream probes on 7q34. Five probes are included for the *SPINK1* gene, detecting exons 2, 3, 4, 5, and a region upstream of exon 1. Eight probes are included detecting all exons of *CTRC* gene. In addition, ten reference probes are included that detect autosomal chromosomal locations. Complete probe sequences and the identity of the genes detected by the reference probes are available online (www.mrcholland.com).

This probemix contains nine quality control fragments generating amplification products between 64 and 105 nt: four DNA Quantity fragments (Q-fragments), two DNA Denaturation fragments (D-fragments), one Benchmark fragment, and one chromosome X and one chromosome Y-specific fragment (see table below). More information on how to interpret observations on these control fragments can be found in the MLPA General Protocol and online at www.mrcholland.com.

| Length (nt) | Name |
|---|---|
| 64-70-76-82 | Q-fragments (only visible with <100 ng sample DNA) |
| 88-96 | D-fragments (low signal indicates incomplete denaturation) |

| | |
|---|---|
| 92 | Benchmark fragment |
| 100 | X-fragment (X chromosome specific) |
| 105 | Y-fragment (Y chromosome specific) |

**MLPA technique**
The principles of the MLPA technique (Schouten et al. 2002) are described in the MLPA General Protocol (www.mrcholland.com).

**MLPA technique validation**
Internal validation of the MLPA technique using 16 DNA samples from healthy individuals is required, in particular when using MLPA for the first time, or when changing the sample handling procedure, DNA extraction method or instruments used. This validation experiment should result in a standard deviation ≤0.10 for all probes over the experiment.

**Required specimens**
Extracted DNA free from impurities known to affect MLPA reactions. For more information please refer to the section on DNA sample treatment found in the MLPA General Protocol.

**Reference samples**
A sufficient number (≥3) of reference samples should be included in each MLPA experiment for data normalisation. All samples tested, including reference DNA samples, should be derived from the same tissue type, handled using the same procedure, and prepared using the same DNA extraction method when possible. Reference samples should be derived from different unrelated individuals who are from families without a history of pancreatitis. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol (www.mrcholland.com).

**Positive control DNA samples**
MRC Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/) have diverse collections of biological resources which may be used as positive control DNA samples in your MLPA experiments. Sample ID numbers NA11949, NA07412, and NA12519 from the Coriell Institute have been tested with this P242-C1 probemix at MRC-Holland and can be used as positive control samples (see table below). The quality of cell lines can change; therefore samples should be validated before use.

| Sample name | Source | Altered target gene/region in P242-C1 | Expected copy number alteration |
|---|---|---|---|
| NA11949 | Coriell Institute | *PRSS1* | Heterozygous deletion affecting all *PRSS1* probes. |
| NA07412 | Coriell Institute | 7q34 region | Heterozygous deletion affecting the probes for *BRAF*, *PRSS1*, and *CASP2*. |
| NA12519 | Coriell Institute | 7q34 region | Homozygous duplication affecting the probes for *BRAF*, *PRSS1*, and *CASP2*. |

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

**Interpretation of results**
The standard deviation of each individual probe over all the reference samples should be ≤0.10 and the final ratio (FR) of each individual reference probe in the patient samples should be between 0.80 and 1.20. When these criteria are fulfilled, the following cut-off values for the FR of the probes can be used to interpret MLPA results for autosomal chromosomes or pseudo-autosomal regions:

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

Note: The term "dosage quotient", used in older product description versions, has been replaced by "final ratio" to become consistent with the terminology of the Coffalyser.Net software. (Calculations, cut-offs and interpretation remain unchanged.) Please note that the Coffalyser.Net software also shows arbitrary borders as part of the statistical analysis of results obtained in an experiment. As such, arbitrary borders are different from the final ratio cut-off values shown here above.

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Sequence changes (e.g. SNVs, point mutations) in the target sequence detected by a probe can be one cause. Incomplete DNA denaturation (e.g. due to salt contamination) can also lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. 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.

P242 specific note - *PRSS1* results:

SALSA MLPA Probemix P242 Pancreatitis contains probes for the *PRSS1* gene. *PRSS1* is located in a genomic region known to be excised during T cell maturation. The apparent copy number for *PRSS1* in a sample will therefore be dependent on the percentage of DNA derived from mature T cells. As a result, the PRSS1 probes show more variability as compared to the other probes present in this probemix.

It is strongly recommended to use selected reference samples, these can be chosen by testing several samples from healthy individuals. These reference samples should be derived from individuals of a similar age to most test samples, from similar test material, and have an intermediate *PRSS1* copy number (ratio 1).

**Even with selected reference samples, it is possible to obtain low or high PRSS1 probe ratios (0.8 or 1.2)** in certain samples due to an unusually high or low percentage of mature T cells, respectively.

Note: Sample types that do not have T cell rearrangements (e.g. cell lines) should not be used as references samples for P242.

**Limitations of the procedure**

- In most populations, the major cause of genetic defects in the *PRSS1, SPINK1,* and *CTRC* genes are small (point) mutations, none of which will be detected by using SALSA MLPA Probemix P242 Pancreatitis.
- 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.

***PRSS1*, *SPINK1*, and *CTRC* 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 SNVs and unusual results (e.g., a duplication of *PRSS1* exons 3 and 5 but not exon 4) to MRC Holland: info@mrcholland.com.

## Table 1. SALSA MLPA Probemix P242-C1 Pancreatitis

| Length (nt) | SALSA MLPA probe | Chromosomal position (hg18)[a] | | | |
|---|---|---|---|---|---|
| | | Reference | *PRSS1* | *SPINK1* | *CTRC* |
| 64-105 | Control fragments – see table in probemix content section for more information | | | | |
| 136 | Reference probe 00662-L00158 | 6p | | | |
| 142 | **PRSS1 probe** 09213-L09483 | | **Exon 4** | | |
| 148 | **CTRC probe** 22402-L32009 | | | | **Exon 1** |
| 160 ¬ | **BRAF probe** 04259-L03624 | | **Upstream** | | |
| 166 | Reference probe 18955-L26262 | 2q | | | |
| 171 | **CTRC probe** 22403-L31592 | | | | **Exon 8** |
| 178 | **PRSS1 probe** 20261-L31886 | | **Exon 5** | | |
| 184 | **SPINK1 probe** 08413-L08297 | | | **Exon 4** | |
| 196 | **CTRC probe** 22404-L31746 | | | | **Exon 6** |
| 202 | Reference probe 05706-L20677 | 3q | | | |
| 208 ¬ | **CASP2 probe** 00552-L28279 | | **Downstream** | | |
| 218 | **CTRC probe** 22405-L31594 | | | | **Exon 4** |
| 223 | **SPINK1 probe** 08412-L28280 | | | **Exon 2** | |
| 229 | Reference probe 10149-L27417 | 18q | | | |
| 235 | **CTRC probe** 22406-L31595 | | | | **Exon 2** |
| 241 | **PRSS1 probe** 08408-L09361 | | **Exon 3** | | |
| 247 | Reference probe 21928-L30731 | 15q | | | |
| 254 | **CTRC probe** 22408-L31597 | | | | **Exon 3** |
| 258 | **CTRC probe** 22407-L31747 | | | | **Exon 7** |
| 265 | **PRSS1 probe** 09210-L10056 | | **Exon 1** | | |
| 280 | Reference probe 13350-L26120 | 9q | | | |
| 295 | **SPINK1 probe** 09215-L28282 | | | **Exon 3** | |
| 304 | **PRSS1 probe** 09211-L28284 | | **Exon 1** | | |
| 310 | Reference probe 15855-L17948 | 11p | | | |
| 319 ¬ | **CASP2 probe** 02051-L01583 | | **Downstream** | | |
| 337 | **PRSS1 probe** 09212-L09482 | | **Exon 2** | | |
| 346 | Reference probe 03874-L03322 | 20q | | | |
| 374 | **CTRC probe** 22409-L31598 | | | | **Exon 5** |
| 391 | **SPINK1 probe** 22581-L31780 | | | **Exon 5** | |
| 409 | Reference probe 10063-L10487 | 8q | | | |
| 427 | **SPINK1 probe** 22582-L31781 | | | **Upstream** | |
| 436 | Reference probe 19646-L26317 | 17p | | | |

[a] See section Exon numbering on page 2 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.

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

## Table 2. P242-C1 probes arranged according to chromosomal location

Table 2a. *PRSS1*

| Length (nt) | SALSA MLPA probe | *PRSS1* exon[a] | Ligation site NM_002769.5 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| 160 ¬ | 04259-L03624 | *BRAF* gene | | TTGGGACACTGA-TATTTCCTGGCT | 1982.6 kb |
| | | | | | |
| | | *start codon* | *14-16 (Exon 1)* | | |
| 265 | 09210-L10056 | Exon 1 | 36 nt before exon 1 | CTGGATCCTCGT-GAGGTATAAAGA | 0.1 kb |
| 304 # | 09211-L28284 | Exon 1 | 53-54 | TGGCAGCTGCTC-GTGAGTATCATG | 1.2 kb |
| 337 # + | 09212-L09482 | Exon 2 | 174-175 | CTCCCTCATCAA-CGAACAGTGGGT | 1.2 kb |
| 241 | 08408-L09361 | Exon 3 | 343-344 | GACATCATGTTA-ATCAAGCTCTCC | 0.7 kb |
| 142 # | 09213-L09483 | Exon 4 | 598-599 | GGCAAGGATTCA-TGTCAGGTGATT | 0.4 kb |
| 178 # | 20261-L31886 | Exon 5 | 722-723 | ACAACTATGTGA-AATGGATTAAGA | 559.4 kb |
| | | *stop codon* | *755-757 (Exon 5)* | | |
| | | | | | |
| 319 ¬ | 02051-L01583 | *CASP2* gene | | TTTCTTACAGTT-GAGCTGTGACTA | 11.2 kb |
| 208 ¬ | 00552-L28279 | *CASP2* gene | | TGGGGTTGACCA-ACAAGATGGAAA | |

Table 2b. *SPINK1*

| Length (nt) | SALSA MLPA probe | *SPINK1* exon[a] | Ligation site NM_003122.5 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *255-257 (Exon 2)* | | |
| 427 | 22582-L31781 | Upstream | 2.2 kb before exon 1 | GCTACCATGTGG-TCAGCACACTAC | 9.9 kb |
| 223 | 08412-L28280 | Exon 2 | 223-224 | GCGGTGCAGTTT-TCAACTGACCTC | 2.0 kb |
| 295 | 09215-L28282 | Exon 3 | 312-313 | TATTTCTAGGTA-ACACTGGAGCTG | 1.5 kb |
| 184 | 08413-L08297 | Exon 4 | 381-382 | GCACCAAGATAT-ATGACCCTGTCT | 3.4 kb |
| 391 | 22581-L31780 | Exon 5 | 461-462 | AAACGCCAGACT-TCTATCCTCATT | |
| | | *stop codon* | *492-494 (Exon 5)* | | |

Table 2c. *CTRC*

| Length (nt) | SALSA MLPA probe | *CTRC* exon[a] | Ligation site NM_007272.3 | Partial sequence[b] (24 nt adjacent to ligation site) | Distance to next probe |
|---|---|---|---|---|---|
| | | *start codon* | *23-25 (Exon 1)* | | |
| 148 | 22402-L32009 | Exon 1 | 39-40 | GGGCATCACTGT-CCTCGCTGCGCT | 1.9 kb |
| 235 | 22406-L31595 | Exon 2 | 4 nt after exon 2 | CCTGGCAGGTAA-GCCTGTGTAGGG | 0.1 kb |
| 254 | 22408-L31597 | Exon 3 | 215-216 | GGACTTTGATTG-CTAGCAACTTCG | 1.9 kb |
| 218 | 22405-L31594 | Exon 4 | 292-293 | GGAAAGAACAAC-CTGGAGGTGGAA | 1.1 kb |
| 374 | 22409-L31598 | Exon 5 | 9 nt after exon 5 | GGAGTGAGTATC-GTCCCTGGCAAA | 1.2 kb |
| 196 | 22404-L31746 | Exon 6 | 661 - intron 6 | TCAGCCTGCAAT-GTGAGTGGCTAG | 0.9 kb |
| 258 | 22407-L31747 | Exon 7 | 749-750 | CCCGGCGGGGCT-GCAACACCCGCA | 0.9 kb |
| 171 | 22403-L31592 | Exon 8 | 830-831 | TGCAGCTGTGAT-TTGTTGCTGGGA | |
| | | *stop codon* | *827-829 (Exon 8)* | | |

[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.

¬ 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.
# This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. 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 gene or pseudogene.
# + This probe's specificity relies on a single nucleotide difference compared to a related gene or pseudogene. As a result: **1)** an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in

the related gene or pseudogene; **2)** an apparent deletion can be the result of gene conversion between *PRSS1* and *PRSS2* in exon 2 (Teich et al. 2005).

**Note:** *PRSS1* is in a genomic region known to be excised during T cell maturation. As a result, the PRSS1 probes show variability based on the percentage of DNA derived from mature T cells. Using selected reference samples is strongly recommended (see page 1).

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

Complete probe sequences are available at www.mrcholland.com.

## Related SALSA MLPA probemixes

| | |
|---|---|
| P091 CFTR | Contains probes for the *CFTR* gene, related to chronic pancreatitis. |

## References


- Koziel D et al. (2015). Genetic mutations in SPINK1, CFTR, CTRC genes in acute pancreatitis. *BMC Gastroenterol*. 15:70.
- Masson E et al. (2008). Association of rare chymotrypsinogen C (CTRC) gene variations in patients with idiopathic chronic pancreatitis. *Hum Genet*. 123:83-91.
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- Németh BC & Sahin-Tóth M (2014). Human cationic trypsinogen (PRSS1) variants and chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol*, 306(6), G466-G473.
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- Schouten JP et al. (2002). Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic Acids Res.* 30:e57.
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- Teich N et al. (2005). Gene conversion between functional trypsinogen genes PRSS1 and PRSS2 associated with chronic pancreatitis in a six-year-old girl. *Hum Mutat*. 25:343-347.
- 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 P242 Pancreatitis


- Cho SM et al. (2016). PRSS1, SPINK1, CFTR, and CTRC Pathogenic Variants in Korean Patients With Idiopathic Pancreatitis. *Ann Lab Med*. 36:555-60.
- Hegyi E et al. (2014). Chronic pancreatitis associated with the p.G208A variant of PRSS1 gene in a European patient. *JOP*. 15:49-52.
- Jancsó Z et al. (2019). Novel pathogenic PRSS1 variant p. Glu190Lys in a case of chronic pancreatitis. *Front Genet*, 10, 46.
- Kujko AA et al. (2017). A novel p.Ser282Pro CPA1 variant is associated with autosomal dominant hereditary pancreatitis. *Gut*. 66:1728-30.
- Rygiel AM et al. (2015). Gene conversion between cationic trypsinogen (PRSS1) and the pseudogene trypsinogen 6 (PRSS3P2) in patients with chronic pancreatitis. *Hum Mutat*. 36:350-6.


| **P242 product history** | |
|---|---|
| *Version* | *Modification* |
| C1 | Eight CTRC probes and two SPINK1 probes have been added. One SPINK1 probe has been removed. One probe has been adjusted in length. Seven reference probes have been replaced. |

| | |
|---|---|
| B3 | One flanking probe has been removed and four reference probes have been replaced. |
| B2 | Three reference probes have been replaced and the control fragments have been adjusted (QDX2). |
| B1 | The *PRSS2* probe at 165 nt has been removed because of its high standard deviation. |
| A1 | First release. |

| **Implemented changes in the product description** |
|---|
| Version C1-03 – 03 August 2023 (04P)<br>- Product description rewritten and adapted to a new template.<br>- Ligation sites of the probes targeting the *SPINK1* gene updated according to new version of the NM_ reference sequence.<br>- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.<br>*Version C1-02 — 23 February 2021 (02P)*<br>- Warning adjusted on page 1, interpretation of results section and below Table 2a regarding PRSS1 probe ratios of ~0.8.<br>- Positive samples NA07412 and NA12519 added to the section Positive control DNA samples.<br>*Version C1-01 — 10 December 2019 (02P)*<br>- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).<br>- Positive control DNA samples section was updated.<br>- Ligation sites of the probes targeting the *PRSS1* gene updated according to new version of the NM_ reference sequence.<br>- Small changes of probe length in Table 1 and 2 in order to better reflect the true lengths of the amplification products. |

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