Product Description SALSA® MLPA® Probemix P272-C1 COL1A2

To be used with the MLPA General Protocol.

Version C1. For complete product history see page 6.

Catalogue numbers:
- **P272-025R:** SALSA MLPA Probemix P272 COL1A2, 25 reactions.
- **P272-050R:** SALSA MLPA Probemix P272 COL1A2, 50 reactions.
- **P272-100R:** SALSA MLPA Probemix P272 COL1A2, 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 P272 COL1A2 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *COL1A2* gene, which is associated with Osteogenesis Imperfecta (OI).

Type I collagen is the most common type of collagen. It is present in almost all connective tissues. This protein consists of three polypeptide chains: two alpha-1 polypeptide chains and one alpha-2 polypeptide chain, which are encoded by the *COL1A1* gene and the *COL1A2* gene, respectively.

OI is a genetic disorder characterised by bone fragility, severe bowing of long bones and low bone mass. The prevalence of this disorder ranges from one per 10,000 to one per 20,000 live births. Severe forms of OI lead to intrauterine fractures and perinatal lethality. Besides bone, other tissues rich in type I collagen are also affected, including skin, ligaments, and tendons. About 90% of the patients diagnosed with OI have mutations in the *COL1A1* or *COL1A2* genes.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1295/.

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 *COL1A2* exon numbering used in this P272-C1 COL1A2 product description is the exon numbering from the RefSeq transcript NM_000089.3, which is identical to the LRG_2 sequence. The exon numbering and NM_ sequence used have been retrieved on 05/2020. 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 P272-C1 COL1A2 contains 43 MLPA probes with amplification products between 130 and 472 nucleotides (nt). This includes 35 probes for the *COL1A2* gene, which targets 35 out of 52 exons of the gene. In addition, eight 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 Osteogenesis Imperfecta. 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 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

Copy number status	Dosage quotient
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 *COL1A2* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P272 COL1A2.
- 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.

***COL1A2* mutation database:** https://oi.gene.le.ac.uk/home.php?select_db=COL1A2. We strongly encourage users to deposit positive results in the Osteogenesis Imperfecta Variant Database of the LOVD gene homepage. 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 *COL1A2* exons 10 and 12 but not exon 11) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P272-C1 COL1A2

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a]	
		Reference	COL1A2
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 00797-L00463	5q31	
136	**COL1A2 probe** 07990-L07771		**Exon 10**
142	**COL1A2 probe** 07999-L07780		**Exon 25**
149	**COL1A2 probe** 07987-L07768		**Exon 5**
154	**COL1A2 probe** 08002-L07783		**Exon 30**
160	**COL1A2 probe** 07992-L07773		**Exon 12**
166	**COL1A2 probe** 08011-L07792		**Exon 41**
172	**COL1A2 probe** 09438-L07776		**Exon 18**
179	**COL1A2 probe** 08015-L07796		**Exon 49**
184	Reference probe 08788-L08812	10q21	
191	**COL1A2 probe** 08006-L21847		**Exon 34**
197	**COL1A2 probe** 07985-L21848		**Exon 2**
203	**COL1A2 probe** 08005-L21849		**Exon 33**
211	**COL1A2 probe** 09437-L21850		**Exon 20**
220	**COL1A2 probe** 08010-L21851		**Exon 40**
229	**COL1A2 probe** 08007-L21852		**Exon 35**
238	**COL1A2 probe** 07997-L07778		**Exon 22**
247	**COL1A2 probe** 08013-L21853		**Exon 44**
257	Reference probe 17408-L21394	3p21	
265	**COL1A2 probe** 08000-L07781		**Exon 27**
274	**COL1A2 probe** 08016-L07797		**Exon 50**
283	**COL1A2 probe** 10185-L22730		**Exon 31**
292	**COL1A2 probe** 07984-L07765		**Exon 1**
301	**COL1A2 probe** 08014-L07795		**Exon 47**
310	**COL1A2 probe** 07993-L07774		**Exon 13**
318	**COL1A2 probe** 08008-L07789		**Exon 37**
337	Reference probe 11444-L19619	1q41	
346	Reference probe 15885-L17978	2p16	
353	**COL1A2 probe** 07994-L07775		**Exon 15**
360	**COL1A2 probe** 08001-L21854		**Exon 29**
369	Reference probe 01580-L21859	22q12	
377	**COL1A2 probe** 07998-L21855		**Exon 23**
385	**COL1A2 probe** 07986-L21856		**Exon 4**
396	**COL1A2 probe** 08004-L22342		**Exon 32**
405	**COL1A2 probe** 07991-L22343		**Exon 11**
411	**COL1A2 probe** 08009-L07790		**Exon 39**
418	**COL1A2 probe** 08012-L07793		**Exon 42**
427	**COL1A2 probe** 08017-L07798		**Exon 52**
436	**COL1A2 probe** 09439-L07770		**Exon 9**
445	Reference probe 01799-L01362	13q14	
454	**COL1A2 probe** 13154-L14442		**Exon 51**
463 Ø	**COL1A2 probe** 13155-L14444		**Intron 6**
472	Reference probe 12029-L12891	6p22	

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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

Table 2. *COL1A2* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	COL1A2 exon[a]	Ligation site NM_000089.3	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*472-474 (exon 1)*		
292	07984-L07765	Exon 1	83-84	GACAACGAGTCA-GAGTTTCCCCTT	3.2 kb
197	07985-L21848	Exon 2	79 nt after exon 2	TGGAAGGGAAGA-AGTTACATTAAT	1.2 kb
385	07986-L21856	Exon 4	594-595	AGAGGACCACGT-GGAGAAAGGGTG	1.2 kb
149	07987-L07768	Exon 5	10 nt after exon 5	GGTAAGGTGTCT-TACGTATTGCTA	1.9 kb
463 Ø	13155-L14444	Intron 6	596 nt after exon 6	TAGTCCTTGAAT-AAACCACTCATT	2.6 kb
436	09439-L07770	Exon 9	858-859	TAGGGTCCTGCA-GGTGCTCGTGGT	0.4 kb
136	07990-L07771	Exon 10	945-944 reverse	TGTGGTCCAACA-ACTCCTCTCTCA	0.5 kb
405	07991-L22343	Exon 11	1007-1008	CTTCAAAGGCAT-TAGGGTGAGCAC	0.5 kb
160	07992-L07773	Exon 12	1023-1024	GGACACAATGGT-CTGGATGGATTG	1.6 kb
310	07993-L07774	Exon 13	1098-1097 reverse	GTTTGACCTGGA-GTTCCATTTTCA	0.5 kb
353	07994-L07775	Exon 15	1209-1208 reverse	TCAAAACTTACA-GCAGGACCCACG	1.2 kb
172	09438-L07776	Exon 18	1377-1378	AATCCTGGAGCA-AACGGCCTTACT	0.7 kb
211	09437-L21850	Exon 20	1547-1548	AGAGAGCGGTAA-CAAGGGTGAGCC	0.6 kb
238	07997-L07778	Exon 22	1702-1703	TTCCTGGAGCTG-ATGGCAGAGCTG	0.2 kb
377	07998-L21855	Exon 23	7 nt after exon 23 reverse	GAATCAGTTTGA-AACTTACTCTGG	1.5 kb
142	07999-L07780	Exon 25	1946-1947	GCCTGGCAACAT-TGGATTCCCTGG	1.1 kb
265	08000-L07781	Exon 27	2053-2054	CTGATGGAAACA-ATGGTGCTCAGG	0.5 kb
360	08001-L21854	Exon 29	2178-2177 reverse	CTTTCTCCTGGT-TTGCCAACTTCA	1.0 kb
154	08002-L07783	Exon 30	2206-2207	TCCATGGTGAGT-TTGGTCTCCCTG	1.2 kb
283	10185-L22730	Exon 31	2287-2288	CTACTGGTCCTA-TTGGAAGCCGAG	1.4 kb
396	08004-L22342	Exon 32	1 nt after exon 32	GGGAGAAAAGGT-ACGTGTTGACCC	0.7 kb
203	08005-L21849	Exon 33	2469-2468 reverse	CCAGGGTTACCA-ATTTCACCTCTG	1.1 kb
191	08006-L21847	Exon 34	19 nt after exon 34	GCATTTTCACTA-AGCCAACAGCAA	0.6 kb
229	08007-L21852	Exon 35	18 nt before exon 35	TTAACAGATTCA-TCTTTGGTCCCA	0.4 kb
318	08008-L07789	Exon 37	10 nt after exon 37 reverse	ACCACCAGTGAA-TTCAACTTACAG	1.2 kb
411	08009-L07790	Exon 39	23 nt before exon 39	GTTGACTGTGGA-ATTCTAATGTGC	1.2 kb
220	08010-L21851	Exon 40	2952-2953	GACCAAGGTCCA-GTTGGCCGAACT	1.3 kb
166	08011-L07792	Exon 41	3086-3087	TGCTCCTGGTAT-TCTGGGTCTCCC	0.8 kb
418	08012-L07793	Exon 42	3219-3220	GGTAGTCCTGGA-GTCAACGGTGCT	0.7 kb
247	08013-L21853	Exon 44	3399-3400	GGCAAACATGGA-AACCGTGGTGAA	1.2 kb
301	08014-L07795	Exon 47	13 nt after exon 47	AGGTGGACTCAA-GAGAAGACAGTT	0.6 kb
179	08015-L07796	Exon 49	3794-3795	CGGTGGTGGTTA-TGACTTTGGTTA	0.7 kb
274	08016-L07797	Exon 50	4139-4140	CTCCAAGGACAA-GAAACACGTCTG	0.6 kb
454	13154-L14442	Exon 51	187 nt before exon 51	TAGGTCTTATGT-TCATCTAGGTAA	1.8 kb
427	08017-L07798	Exon 52	4989-4990	GATCCACATTGT-TAGGTGCTGACC	
		stop codon	*4570-4572 (exon 52)*		

Ø Intron probe. Only included to help determine the extent of a deletion/duplication. Copy number alterations of only this probe are of unknown clinical significance.

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.

Related SALSA MLPA probemixes

P271 COL1A1 Contains probes for the *COL1A1* gene, which is also involved in osteogenesis imperfecta.
P452 PLS3 Contains probes for the *PLS3* gene, which is also involved in osteogenesis imperfecta.

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 P272 COL1A2

- Andersson K et al. (2017). Mutations in COL1A1 and COL1A2 and dental aberrations in children and adolescents with osteogenesis imperfecta–A retrospective cohort study. *PLoS One*, 12(5).
- Kanno J et al. (2018). Responsiveness to pamidronate treatment is not related to the genotype of type I collagen in patients with osteogenesis imperfecta. *J bone miner metab*, 36(3), 344-351.
- Li L et al. (2019). Genotypic and phenotypic characterization of Chinese patients with osteogenesis imperfecta. *Hum mutat*, 40(5), 588-600.
- Lindahl K et al. (2015). Genetic epidemiology, prevalence, and genotype–phenotype correlations in the Swedish population with osteogenesis imperfecta. *Eur J Hum Genet* 23(8): 1042-1050.
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- Takagi M et al. (2014). Osteogenesis imperfecta IIC caused by a novel heterozygous mutation in the C-propeptide region of COL1A1. *Hum genome var*, 1(1), 1-3.
- Takagi M et al. (2015). Severe osteogenesis imperfecta caused by double glycine substitutions near the amino-terminal triple helical region in COL1A2. *Am J Med Genet Part A* , 167(7), 1627-1631.
- Vandersteen A et al. (2014). Four patients with Sillence type I osteogenesis imperfecta and mild bone fragility, complicated by left ventricular cardiac valvular disease and cardiac tissue fragility caused by type I collagen mutations. *Am J Med Genet Part A* 164(2): 386-391.

P272 Product history	
Version	*Modification*
C1	One target probe has been removed.
B2	Three reference probes have been replaced and QDX2 fragments have been added.
B1	One *COL1A2* probe has been replaced, two *COL1A2* probes, two reference probes, and two control fragments have been added.
A1	First release.

Implemented changes in the product description

Version C1-01 — 08 July 2020 (02P)
- Product description rewritten and adapted to a new template.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.

Version 14 – 14 October 2016 (55)
- Product description adapted to a new product version (version number changed, lot number added, small changes in Table 1 and Table 2, new picture included).
- Several minor errors in Table 1 and Table 2 were corrected.
- Small changes of probe lengths in Table 1 and 2 in order to better reflect the true lengths of the amplification products.
- Various minor textual changes.

Version 13 – 16 January 2015 (54)
- Warning added in Table 1, 328 nt probe 07988-L14804.
- Various minor textual changes on page 1.
- Corrected text on data analysis.
- Corrected text in the figure legends.

Version 12 (48)
- Chromosomal position of 256 nt reference probe (17408-L21394) added to Table 1.

Version 11 (48)
- Electropherogram pictures using the new MLPA buffer (introduced in December 2012) added.

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