Product Description SALSA® MLPA® Probemix P292-B2 PCDH15

To be used with the MLPA General Protocol.

Version B2. As compared to version B1, four reference probes have been replaced. For complete product history see page 7.

Catalogue numbers:
- **P292-025R:** SALSA MLPA Probemix P292 PCDH15, 25 reactions.
- **P292-050R:** SALSA MLPA Probemix P292 PCDH15, 50 reactions.
- **P292-100R:** SALSA MLPA Probemix P292 PCDH15, 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 P292 PCDH15 is a **research use only (RUO)** assay for the detection of deletions or duplications in the *PCDH15* gene, which is associated with Usher syndrome.

Usher syndrome is a heterogeneous autosomal recessive disorder characterised by hearing loss and retinitis pigmentosa. Three clinical types (USH1, -2, and -3) are defined with respect to the degree and progression of hearing loss and the presence or absence of vestibular dysfunction. Usher syndrome type I (USH1) is the most disabling form, while USH2 is the most common of the three types of Usher syndrome. The following six genes have been identified for USH1: *MYO7A* (Myosin VIIA, USH1B), *USH1C* (Usher syndrome 1C), *CDH23* (Cadherin related 23, USH1D), *PCDH15* (Protocadherin related 15, USH1F), *USH1G* (Usher syndrome 1G) and *CIB2* (Calcium and integrin binding family member 2, USH1J). Mutations in the *PCDH15* gene are associated with Usher syndrome type 1F (USH1F). The gene has been shown to be particularly prone to large rearrangements (Roux et al. 2011). The *PCDH15* gene (33 exons) spans ~1 Mb of genomic DNA and is located on 10q21.1, ~55 Mb from the p-telomere. The gene is a member of the cadherin superfamily and encodes for an integral membrane protein that mediates calcium-dependent cell-cell adhesion.

More information is available at https://www.ncbi.nlm.nih.gov/books/NBK1265/.

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 *PCDH15* exon numbering used in this P292-B2 PCDH15 product description is the exon numbering from the RefSeq transcript NM_033056.4, which is identical to the NG_009191.3 sequence. The exon numbering and NM_ sequence used have been retrieved on 06/2020. 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 P292-B2 PCDH15 contains 42 MLPA probes with amplification products between 130 and 463 nucleotides (nt). This includes 33 probes for the *PCDH15* gene: one probe for each exon with the exception of exon 31 and 32, and a second probe for both exon 1 and 2. In addition, nine 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 Usher syndrome. More information regarding the selection and use of reference samples can be found in the MLPA General Protocol.

Positive control DNA samples: MRC-Holland cannot provide positive DNA samples. Inclusion of a positive sample in each experiment is recommended. Coriell Institute (https://catalog.coriell.org) and Leibniz Institute DSMZ (https://www.dsmz.de/home.html) have a diverse collection of biological resources which may be used as a positive control DNA sample in your MLPA experiments. Sample ID number NA11672 from the Coriell Institute has been tested with this P292-B2 probemix at MRC-Holland and can be used as a positive control sample to detect a heterozygous deletion of all exons of *PCDH15*. The quality of cell lines can change; therefore samples should be validated before use.

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

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

Copy number status	Dosage quotient
Normal	0.80 < DQ < 1.20
Homozygous deletion	DQ = 0
Heterozygous deletion	0.40 < DQ < 0.65
Heterozygous duplication	1.30 < DQ < 1.65
Heterozygous triplication/Homozygous duplication	1.75 < DQ < 2.15
Ambiguous copy number	All other values

- Arranging probes according to chromosomal location facilitates interpretation of the results and may reveal more subtle changes such as those observed in mosaic cases. Analysis of parental samples may be necessary for correct interpretation of complex results.
- False positive results: Please note that abnormalities detected by a single probe (or multiple consecutive probes) still have a considerable chance of being a false positive result. Incomplete DNA denaturation (e.g. due to salt contamination) can lead to a decreased probe signal, in particular for probes located in or near a GC-rich region. The use of an additional purification step or an alternative DNA extraction method may resolve such cases. Additionally, contamination of DNA samples with cDNA or PCR amplicons of individual exons can lead to an increased probe signal (Varga et al. 2012). Analysis of an independently collected secondary DNA sample can exclude these kinds of contamination artefacts.
- Normal copy number variation in healthy individuals is described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home. Users should always consult the latest update of the database and scientific literature when interpreting their findings.
- Not all abnormalities detected by MLPA are pathogenic. In some genes, intragenic deletions are known that result in very mild or no disease (as described for *DMD* by Schwartz et al. 2007). For many genes, more than one transcript variant exists. Copy number changes of exons that are not present in all transcript variants may not have clinical significance. Duplications that include the first or last exon of a gene (e.g. exons 1-3) might not result in inactivation of that gene copy.
- Copy number changes detected by reference probes or flanking probes are unlikely to have any relation to the condition tested for.
- When running MLPA products, the capillary electrophoresis protocol may need optimization. False results can be obtained if one or more peaks are off-scale. For example, a duplication of one or more exons can be obscured when peaks are off-scale, resulting in a false negative result. The risk on off-scale peaks is higher when probemixes are used that contain a relatively low number of probes. Coffalyser.Net software warns for off-scale peaks while other software does not. If one or more peaks are off-scale, rerun the PCR products using either: lower injection voltage / injection time settings, or a reduced amount of sample by diluting PCR products.

Limitations of the procedure:

- In most populations, the major cause of genetic defects in the *PCDH15* gene are small (point) mutations, most of which will not be detected by using SALSA MLPA Probemix P292 PCDH15.
- 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.

***PCDH15* mutation database:** https://databases.lovd.nl/shared/genes/PCDH15. 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 *PCDH15* exons 4 and 6 but not exon 5) to MRC-Holland: info@mlpa.com.

Table 1. SALSA MLPA Probemix P292-B2 PCDH15

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)[a] Reference	*PCDH15*
64-105	Control fragments – see table in probemix content section for more information		
130	Reference probe 19616-L26704	4p13	
136 *	Reference probe 06972-L06552	17p13	
142	**PCDH15 probe** 08765-L08805		**Exon 18**
148	**PCDH15 probe** 08767-L11764		**Exon 20**
154	**PCDH15 probe** 08750-L08790		**Exon 3**
166	**PCDH15 probe** 08769-L11317		**Exon 22**
172	**PCDH15 probe** 11542-L12284		**Exon 26**
178	**PCDH15 probe** 08753-L08793		**Exon 6**
184	**PCDH15 probe** 08788-L08812		**Exon 25**
190	**PCDH15 probe** 12409-L29450		**Exon 23**
196	**PCDH15 probe** 08747-L11148		**Exon 1**
202	**PCDH15 probe** 08764-L11316		**Exon 17**
208	Reference probe 08637-L08653	3q26	
214	**PCDH15 probe** 08757-L08797		**Exon 10**
220	Reference probe 08049-L26090	5p15	
226	**PCDH15 probe** 08760-L11318		**Exon 13**
232	**PCDH15 probe** 08751-L08791		**Exon 4**
238	**PCDH15 probe** 08791-L08815		**Exon 28**
247 *	Reference probe 11138-L16379	13q22	
256	**PCDH15 probe** 08758-L08798		**Exon 11**
265	**PCDH15 probe** 08755-L08795		**Exon 8**
274	**PCDH15 probe** 08766-L08806		**Exon 19**
283	**PCDH15 probe** 08749-L08789		**Exon 2**
292	**PCDH15 probe** 08790-L11322		**Exon 27**
301 *	Reference probe 17452-L21208	12p13	
310	**PCDH15 probe** 08754-L08794		**Exon 7**
319	**PCDH15 probe** 08761-L08801		**Exon 14**
328	**PCDH15 probe** 08768-L11325		**Exon 21**
346	**PCDH15 probe** 18077-L22476		**Exon 9**
355	**PCDH15 probe** 20582-L21773		**Exon 16**
364	**PCDH15 probe** 08748-L08788		**Exon 2**
373	**PCDH15 probe** 08759-L11326		**Exon 12**
382	**PCDH15 probe** 21013-L11327		**Exon 1**
391	**PCDH15 probe** 08792-L08816		**Exon 29**
400	**PCDH15 probe** 08795-L08819		**Exon 33**
409 *	Reference probe 13405-L31765	6q12	
418	**PCDH15 probe** 08762-L08802		**Exon 15**
427	**PCDH15 probe** 08787-L11328		**Exon 24**
436	**PCDH15 probe** 08752-L11967		**Exon 5**
445	**PCDH15 probe** 08793-L08817		**Exon 30**
454	Reference probe 07520-L07182	14q24	
463	Reference probe 14308-L15978	15q13	

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

* New in version B2.

Table 2. *PCDH15* probes arranged according to chromosomal location

Length (nt)	SALSA MLPA probe	*PCDH15* exon[a]	Ligation site NM_033056.4	Partial sequence[b] (24 nt adjacent to ligation site)	Distance to next probe
		start codon	*336-338 (Exon 2)*		
382	21013-L11327	Exon 1	19 nt before exon 1	AGGGCAGACAGA-TTCCTGCTAGCA	0.1 kb
196	08747-L11148	Exon 1	49-50	TCTCAGAACGTG-GAGAATGATGGT	136.9 kb
364	08748-L08788	Exon 2	339-340	GACAGAAGATGT-TTCGACAGTTTT	0.1 kb
283	08749-L08789	Exon 2	425-426	CAGTATGATGAT-GGTAAGGTTGCT	136.3 kb
154	08750-L08790	Exon 3	455-456	GGAGGACCACCA-GCTACCATAGTT	148.9 kb
232	08751-L08791	Exon 4	519-520	ACAACATGCTGA-TCAAAGGGACTG	9.7 kb
436	08752-L11967	Exon 5	685-686	CATTGTGGTGCA-GGTCCAGTGCAT	22.8 kb
178	08753-L08793	Exon 6	839-840	ACAATATTCACA-GGATTTTCAGGA	16.8 kb
310	08754-L08794	Exon 7	952-953	CACCTTTGAAAT-TCCCCTAATGTT	12.3 kb
265	08755-L08795	Exon 8	1146-1147	CTTGTGTCCTTG-TGCCAAACACTC	80.5 kb
346	18077-L22476	Exon 9	1273-1274	GGACCGGAATAT-TCAACCGCCATC	22.9 kb
214	08757-L08797	Exon 10	1361-1362	CATATGCATCCT-AGGACAGCAGAA	18.2 kb
256	08758-L08798	Exon 11	1542-1543	GTTATCAAGGCT-ATATCCTGGAAT	10.6 kb
373	08759-L11326	Exon 12	1689-1690	ACACCTCAGTCT-TCACCGTCACAC	1.7 kb
226	08760-L11318	Exon 13	1815-1816	GTGAGCCAGTCA-TCGTCAATATTC	30.3 kb
319	08761-L08801	Exon 14	1983-1984	ATGAAATCCTTG-TTGGGGCTCAGG	20.3 kb
418	08762-L08802	Exon 15	2152-2153	TATTGAAGTGCT-TCCACCAAATAA	43.0 kb
355	20582-L21773	Exon 16	2282-2283	GACTCAATAACA-TATGCCATTGAG	10.6 kb
202	08764-L11316	Exon 17	2378-2379	AGGGAAAGCACT-GATCGCTACATT	12.6 kb
142	08765-L08805	Exon 18	2517-2518	ATTTATCTGTGG-TGGAAGAAGAAG	43.9 kb
274	08766-L08806	Exon 19	2810-2811	TCAACATACACT-GTCCTTGTTGAA	2.6 kb
148	08767-L11764	Exon 20	2894-2895	GCAAATGTGTCT-TACCGGATAAGA	24.7 kb
328	08768-L11325	Exon 21	3149-3150	GTGGCTCCGGAT-GCAGTCAAGGGT	33.8 kb
166	08769-L11317	Exon 22	3233-3234	GTGAGGTATAGA-GTAGATGATGTA	2.1 kb
190	12409-L29450	Exon 23	3409-3410	AGTGAAGATTCT-TGTCTTACATCC	18.8 kb
427	08787-L11328	Exon 24	3487-3488	ACTTGCCACCAA-AGGGACCATGGT	2.0 kb
184	08788-L08812	Exon 25	3619-3620	GAATGGACCTCT-GGATTATGAGAC	35.6 kb
172	11542-L12284	Exon 26	3788-3789	TTCTACATCGGA-GGTGTATCTGAA	36.6 kb
292	08790-L11322	Exon 27	3984-3985	TGAGGAGATCCT-ACTTCAAGTTTC	9.5 kb
238	08791-L08815	Exon 28	4077-4078	ATCAGCTGGATA-TGCAAGTCATTG	16.8 kb
391	08792-L08816	Exon 29	4196-4197	GTCGTAGTGGAG-TCCATTGGAGCT	9.0 kb
445	08793-L08817	Exon 30	4387-4388	AGGAGGACGCAT-TCTGGAGATCCG	8.2 kb
	No probe	Exon 31			
	No probe	Exon 32			
400	08795-L08819	Exon 33	4742-4743	AGCAGGGGAAAT-AACTCAGTCTCA	
		stop codon	*6201-6203 (Exon 33)*		

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

- P361 USH2A mix 1 and P362 USH2A mix 2: contain probes for the *USH2A* gene, involved in Usher syndrome type II.

References

- Roux A et al. (2011). Four-year follow-up of diagnostic service in USH1 patients. *Invest Ophthalmol Vis Sci.* 52(7):4063-4071.
- 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 P292 PCDH15

- Aller E et al. (2010). Identification of large rearrangements of the PCDH15 gene by combined MLPA and a CGH: large duplications are responsible for Usher syndrome. *Invest Ophthalmol Vis Sci*. 51(11):5480-5485.
- Aparisi MJ et al. (2014). Targeted next generation sequencing for molecular diagnosis of Usher syndrome. *Orphanet J Rare Dis*. 9:168.
- Blanco-Kelly F et al. (2015). Clinical aspects of Usher syndrome and the USH2A gene in a cohort of 433 patients. *JAMA Ophthalmol*. 133.2:157-164.
- Dad S et al. (2015). Partial USH2A deletions contribute to Usher syndrome in Denmark. *Eur J Hum Genet*. 23(12):1646-1651.
- Neuhaus C et al. (2017). Next-generation sequencing reveals the mutational landscape of clinically diagnosed Usher syndrome: copy number variations, phenocopies, a predominant target for translational read-through, and PEX26 mutated in Heimler syndrome. *Mol Genet Genomic Med*. 5(5):531-552.
- Roux A et al. (2011). Four-year follow-up of diagnostic service in USH1 patients. *Invest Ophthalmol Vis Sci*. 52(7):4063-4071.

P292 Product history	
Version	*Modification*
B2	Four reference probes have been replaced.
B1	Five reference probes have been replaced and one has been added, one probe for *PCDH15* has been removed and several probe lengths have been adjusted.
A2	The 88 and 96 control fragments have been replaced (QDX2).
A1	First release.

Implemented changes in the product description

Version B2-01 — 13 July 2020 (02P)
- Product description rewritten and adapted to a new template.
- Product description adapted to a new product version (version number changed, changes in Table 1 and Table 2).
- Ligation sites of the probes targeting the *PCDH15* gene updated according to new version of the NM_ reference sequence.

Version 07 – 05 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).
- Various minor textual and layout changes.
- Exon numbering of the *PCDH15* gene has been changed.
- Reference added on page 2.

Version 06 – 07 August 2015 (48)
- Electropherogram picture(s) using the old MLPA buffer (replaced in December 2012) removed.

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