Product Description SALSA® MLPA® Probemix P095-A4 Aneuploidy

To be used with the MLPA General Protocol.

Version A4. For complete product history see page 7.

Catalogue numbers:
- **P095-025R:** SALSA MLPA probemix P095 Aneuploidy, 25 reactions.
- **P095-050R:** SALSA MLPA probemix P095 Aneuploidy, 50 reactions.
- **P095-100R:** SALSA MLPA probemix P095 Aneuploidy, 100 reactions.

To be used in combination with a SALSA MLPA reagent kit, available for various number of reactions. MLPA reagent kits are either provided with FAM or Cy5.0 dye-labelled PCR primer, suitable for Applied Biosystems and Beckman 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.

Intended use: This SALSA MLPA probemix P095 Aneuploidy is an in vitro diagnostic (IVD)[1] or research use only (RUO) assay for the detection of DNA copy number of eight DNA sequences on each of four human chromosomes (13, 18, 21, X) and four sequences on chromosome Y as a means to detect an aneuploidy for one of these chromosomes in prenatal and postnatal DNA samples. Purified DNA from prenatal samples should be from (1) (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (2) (un)cultured chorionic villi free from maternal contamination, or (3) fetal blood. Purified DNA from postnatal samples should be from blood or buccal swabs. This probemix can be used for initial diagnosis or confirmation of results from other techniques. This probemix cannot distinguish between normal females (46,XX) and triploidy females (69,XXX). The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

[1]Please note that this probemix is for In Vitro Diagnostic use (IVD) in the countries specified at the end of this product description. In all other countries, the product is for Research Use Only (RUO).

Clinical background: Germline aneuploidy of a complete chromosome usually results in a miscarriage. The most common cases of an abnormal chromosome copy number at birth concern the presence of an extra chromosome 13, 18 or 21, each resulting in mental retardation and other disorders (Patau, Edwards, Down syndromes), or the presence of an extra or missing sex chromosome (e.g. X0, XXY, XYY) - with much less severe consequences (Turner, Triple X, Klinefelter, and XYY syndromes). Full trisomies account for the majority of cases, while a minority results from partial chromosome duplications or mosaicism.

Methods used to detect aneuploidies include karyotyping, FISH, quantitative PCR (in particular QF-PCR of short tandem repeats), MLPA, (array)-CGH, and high throughput sequencing. Aneuploidies can be diagnosed prenatally in samples from amniotic fluid, chorionic villi, fetal blood or from free circulating fetal DNA in the maternal plasma (NIPD). For analysis of postnatal samples, blood or buccal swab derived DNA is commonly used.

More information on aneuploidies is available in http://en.wikipedia.org/wiki/Aneuploidies. See below for several publications on probemix P095 Aneuploidy and several reviews comparing the different methods for aneuploidy testing.

Probemix content: This SALSA MLPA probemix P095 Aneuploidy contains 36 MLPA probes with amplification products between 136 and 454 nt: eight probes for each chromosome 13, 18, 21, and X; and four probes for the Y chromosome. More information is present in Table 2 of this product description.

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 or 96 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 male DNA samples (20 ng each) 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: For prenatal use: extracted DNA from (1) (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (2) (un)cultured chorionic villi free from maternal contamination, or (3) fetal blood. For postnatal use: purified DNA from blood or buccal swabs. DNA samples tested should be free from impurities know to affect MLPA reactions and contain between 20-250 ng fetal DNA for optimal results. The use of lower amounts of sample DNA results in higher variation. For more information, see MLPA General Protocol and the www.mlpa.com website section on DNA sample treatment.

Reference samples: 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 **male** individuals without developmental delay or congenital abnormalities. A minimum of three male reference samples should be included in the experiment. 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 Biobank (https://www.coriell.org) and 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.

Performance characteristics: Several studies have reported a diagnostic and analytical sensitivity and specificity >99% for the detection of chromosomes 13, 18, 21, X and Y aneuploidies (e.g. Boormans et al. 2010, Gerdes et al. 2008, Kooper et al. 2008, and Van Opstal et al. 2009).

Analytical performance can be compromised by: SNPs or other polymorphisms (e.g. indels) in the DNA target sequence, impurities in the DNA sample, incomplete DNA denaturation, the use of insufficient or too much sample DNA, the use of insufficient or unsuitable reference samples, problems with capillary electrophoresis or a poor data normalisation procedure and other technical errors. The MLPA General Protocol contains technical guidelines and information on data evaluation/normalisation.

Data analysis: Coffalyser.Net software must 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: In P095-A4, all autosomal probes detecting loci on chromosomes 13, 18, and 21 are set as reference probes. In full trisomy cases, the values for all eight reference probes detecting the same chromosome will therefore be increased. During intra-sample normalisation, each probe signal is compared with each of the 24 reference probe signals. The Coffalyser.Net software neutralizes the effect of an increased value for 8 out of 24 reference probes in trisomy samples by calculating the median of the 24 normalised values of each probe. The expected Dosage Quotient (DQ)-values for various genotypes as determined by Coffalyser.Net software can be found in Table 1 below.

Table 1. P095 Dosage Quotients expected in aneuploidy and normal cases when using all chromosome 13, 18, and 21 probes as reference probes and using 46,XY as reference samples.

Sample type		chr. 13 probes	chr. 18 probes	chr. 21 probes	chr. X probes	chr. Y probes
46,XY	Normal male	1	1	1	1	1
46,XX *	Normal female	1	1	1	2	0
47,XY,+13	Patau syndrome	1.5	1	1	1	1
47,XY,+18	Edwards syndrome	1	1.5	1	1	1
47,XY,+21	Down syndrome	1	1	1.5	1	1
45,X0	Turner syndrome	1	1	1	1	0
47,XXX	Triple X syndrome	1	1	1	3	0
47,XXY	Klinefelter syndrome	1	1	1	2	1
47,XYY	XYY syndrome	1	1	1	1	2
69,XXX *	Triploidy female	1	1	1	2	0
69,XXY	Triploidy male	1	1	1	1.33	0.67
69,XYY	Triploidy male	1	1	1	0.67	1.33

* MLPA cannot distinguish between 69,XXX and 46,XX samples.

For copy number changes of a complete chromosome (majority of cases):
In theory, all probes targeting a certain chromosome should give approximately the same DQ-value, also in cases of aneuploidy. In practice, however, certain variables such as SNPs, sample impurities etc., can affect this. Based on Van Opstal et al. (2009), we recommend the following rules for determining copy number status:

1. A trisomy for a particular chromosome should be considered when the following two criteria are met:
   I. At least 4 out of 8 probe ratios for a certain chromosome are ≥ 1.30 AND
   II. At least 6 out of 8 probes ratios for that chromosome are ≥ 1.20
2. Monosomy X should be considered if the probe ratios for X chromosome probes are within the range of those of normal males and Y signals are absent.
3. 47,XXY should be considered if probe ratios for Y chromosome probes are within the range for those of normal males and X chromosome probes are ~2 times those of normal males.
4. 47,XYY should be considered if probe ratios for Y chromosome probes are ~2 times those of normal males and X chromosome probes are within the range of those of normal males.
5. 47,XXX should be considered if the relative probe signals for X chromosome probes are ~2.5-3 times those of normal males and Y signals are absent.

For partial chromosome gain or mosaic cases (minority of cases):

1. In case a partial chromosome gain has occurred, a duplication of a specific region will result in a DQ > 1.30 of two or more of the corresponding probes. Additional studies should be used to confirm and interpret the result. As the P095 probe sequences have been selected based on their location on a specific chromosome, not on being located in a specific gene, we recommend to disregard copy number alterations detected by a single probe. Copy number changes detected by only a single probe may be a false positive result due to e.g. a mutation or polymorphism that prevents ligation of the MLPA probe or that destabilizes the binding of a probe oligonucleotide to the sample DNA.
2. We recommend a follow-up study for samples in which several probes for a certain chromosome are marked as having a statistically abnormal probe value by Coffalyser.Net analysis. The use of a 1.30 cut-off value to distinguish a normal from a trisomy result does not allow detection of mosaic samples. As an example, all chromosome 21 specific probes may have a ratio between 1.10 and 1.20 in a mosaic sample with 30% trisomy 21 cells. Coffalyser.Net analysis may identify such mosaic samples when the experiment was performed well.

The presence of salt or other impurities can lead to apparent deletions or duplications detected by one or more probes. The use of an alternative DNA extraction method may resolve such cases. Finally, a large number of copy number alterations in healthy individuals have been described in the database of genomic variants: http://dgv.tcag.ca/dgv/app/home.

Limitations of the procedure:

- For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
- For use on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and foetus have been described due to maternal contamination, postzygotic nondisjunction, postzygotic isochromosome formation, mosaic situations, and complications in DNA sampling in twin pregnancies (Van den Berg et al. 2006).
- 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 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.
- Not all abnormalities detected by MLPA are pathogenic. Probe sequences have been selected based on their location on a specific chromosome, not on being located in a specific gene. Copy number changes detected by a single probe are in many cases benign.
- MLPA cannot distinguish between normal females (46,XX) and triploid females (69,XXX).
- This probemix is not intended to be used for preimplantation genetic diagnosis.

Confirmation of results: Confirmation of results can be performed by karyotyping, FISH, quantitative PCR (STR analysis), (array)-CGH, or low coverage whole genome DNA sequencing.

Table 2. SALSA MLPA Probemix P095-A4 Aneuploidy

Length (nt)	SALSA MLPA probe	Chromosomal position (hg18)	Location (hg18)	Partial sequence[a] (24nt adjacent to ligation site)
64-105	Control fragments – see table in probemix content section for more information			
136 «	00815-L00333	21q22.13	21-037.044	GATTCTTGAAAG-GTGTAGGTTTGA
142	02127-L01638	18q21.2	18-046.827	GCATTGTGCATA-GTTTGATGTGCC
148	00798-L00316	13q32.1	13-094.526	ATCCGGGCATAC-AAAGCAGAAGAG
154 «	00652-L00637	Xq12	X-066.682	CATGCAACTCCT-TCAGCAACAGCA
160	02153-L00596	Yp11.31	Y-002.715	GCACTGAAAGCT-GTAACTCTAAGT
166	00813-L00636	21q21.1	21-021.726	AGAGCTGTCGCA-GACCACGGCCAA
172	00808-L00326	18q21.32	18-055.721	CTCAGGAGATTT-GGAGACAAACTG
178	00799-L00317	13q13.3	13-035.914	GTATTCATTAAG-TGAAATTGTGCC
184 Δ	02155-L01607	Xq23	X-108.774	TAAGCCCAGAGC-CATGGACCCCTG
193	02152-L00592	Yp11.31	Y-002.715	CAACAGGTTGTA-CAGGGATGACTG
202	02115-L01605	21q21.1	21-016.173	CTGTGAGCGATT-TGCCCGAATCAT
211	02845-L02275	18q11.2	18-021.892	CATGCCTTCAGA-TGGAATGGTAGG
220	00582-L00147	13q14.2	13-047.952	GTGGACACTGTG-TACACCTCTGGA
229 «	02898-L02369	Xp21.3	X-024.935	GGCTGATGAAAG-CTGGGTGTCGGA
240	01071-L00464	Yq11.221	Y-014.101	CTTCGGTAGCTT-AAGTCTTTGCCT
247	00816-L00334	21q11.2	21-014.675	GATATGAAAGCG-TAGAGCTGGCAG
255	00807-L00325	18q23	18-075.328	ACCAGCCCCGTT-CACGTCAGTTTC
265	02125-L01636	13q21.33	13-071.154	AGAGGACTCAAA-GTGTCACCTCCC
274 Δ	02904-L02370	Xp11.4	X-038.420	TGTGGTGTGCAG-AACTACACCAAC
283	04189-L03982	Yp11.31	Y-002.889	TCATAGAGGAGG-ATGTTCAGTGCT
291	02116-L01604	21q22.11	21-031.961	AATAGACACATC-GGCCACACCATC
301	00811-L00329	18p11.32	18-000.663	CTTCGAAAAGTT-GAGAAAATTGAT
310	00871-L00461	13q34	13-110.756	CTGGTTGTGTGG-ACTCCTTAATGC
319 ¥	00819-L28681	Xq28	X-152.791	AACAGCAACTTT-GCTCAGAGGTTC
337	00812-L00330	21q21.3	21-026.192	AGATGGATGCAG-AATTCCGACATG
346	00810-L00328	18q21.33	18-059.714	TGGGTTCATGCA-GCAGATCCAGAA
355	00452-L00360	13q13.1	13-031.829	GCGTCTTTCCAC-AGCCAGGCAGTC
364	02906-L02300	Xp22.12	X-020.137	AAGGAAGGACAT-GAAAAGGCAGAT
382	02834-L02265	21q22.3	21-042.660	CCACCATGGAGA-ACAAGGTGATCT
391	02846-L02276	18q11.2	18-021.873	ATGGGAATGATG-GGTCAAGTTAAC
400	00801-L00639	13q14.2	13-049.577	GAAGAACAGAAC-CTTCAGGAATTG
409 ¥	00820-L28679	Xq26.1	X-129.093	TATTGGTCTTGT-GGACAGTAGTTT
427	00817-L00335	21q22.11	21-031.425	TGGTCCTTGTGT-ATAAAGATGGTT
436	02847-L02277	18p11.21	18-013.875	AGTAACCCCTAC-TGCGCCTGCTAC
445 «	00802-L00320	13q34	13-110.170	GCTACGAGCGCT-TCAGTCGCGAGA
454 ¥	01388-L28680	Xp21.2	X-031.108	GCTTTTTTTCTG-GTCGAGTTGCAA

(a) Only partial probe sequences are shown. Complete probe sequences are available at www.mlpa.com. Please notify us of any mistakes: info@mlpa.com.

¥ Changed in version A4. A single nucleotide outside the hybridising region is replaced. No change in length, no change in sequence detected.

Δ There have been several reports about the 184 nt and 274 nt probe being variable; both hybridise in a region for which copy number variation in healthy individuals has been described.

« 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.

Related SALSA MLPA probemixes

P036 and P070 Subtelomeres	These two products contain one probe for each of the 41 different subtelomeric regions and 5 probes near the centromeric regions of the five acrocentric chromosomes. All probes in P036 are different from the probes in P070. We strongly recommend to use both P036 and P070 (or P069) on each sample when subtelomeric abnormalities are studied.
P245 Microdeletion	Probes for 23 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
P064 Microdeletion	Probes for 15 different microdeletion syndromes; can be used for primary screening of microdeletion syndromes.
P106 MRX	Probes for several genes involved in X-linked mental retardation.
P181 Centromeres	Contains one probe for each of the 41 different centromeric regions and an extra probe near the centromeric region of each of the five acrocentric chromosomes, resulting in two probes for each chromosome.
P182 Centromeres	Similar to P181 centromeres, but all probes are different from the P181 probes.
P365 Telomeres	Probes for the 7p, 16p and **21q** subtelomeric regions + the 15qcen and **21qcen** regions.
P249 Telomeres	Probes for the 17p, **18p**, 19p, and 20p subtelomeric regions.
P291 Telomeres	Probes for the **13q**, 14q, 15q, and 16q subtelomeric regions.
P320 Telomeres	Probes for the 17q, **18q**, 19q, and 20q subtelomeric regions.

More probemixes are available for specific syndromes, including RETT(-like) syndrome, DiGeorge, Prader-Willi/Angelman, Lissencephaly, Canavan, Williams syndrome and many more. Please see our website.

References

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Selected publications using SALSA® MLPA® Probemix P095 Aneuploidy

(Also included are articles for MLPA probemix P001 which is an earlier version of P095)

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- Chen CP et al. (2011). Rapid aneuploidy diagnosis by multiplex ligation-dependent probe amplification and array comparative genomic hybridization in pregnancy with major congenital malformations. *Taiwan J Obstet Gynecol* 50:85-94.
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P095 Product history

Version	*Modification*
A4	This probemix is now suitable for use with a DNA input amount between 20-250 ng. In three probes the first nucleotide of the LPO was adjusted. No change in length or in the sequence detected by the probes.
A3	DNA denaturation control fragments at 88 and 96 nts replaced (QDX2).
A2	Four control fragments added at 88, 96, 100, and 105 nts (QDX1).
A1	First release P095. Contains 18 probes that were present in P001 and 18 new probes.
P001	First release P001.

Implemented changes in the product description

Version A4-03 – 17 June 2020 (04)
- Colombia, Israel and Morocco added as countries with IVD status.

Version A4-02 – 14 March 2018 (04)
- Product description adapted to a new template.
- Updated limitations of the procedure chapter.
- Interpretation of results for trisomy detection updated.
- Salt sensitive probes updated in Table 2.
- References to the related SALSA MLPA probemixes P069 Subtelomeres and P358 Telomeres removed, because these probemixes will be discontinued (in Q2 2018).

Version A4-01 – 21 September 2016 (03)
- Product description restructured and adapted to a new template.

Version 22 – 19 June 2015
- Product description adapted to a new product version (version number changed, small changes in Table 2, new picture included).

- The recommended minimum amount of sample DNA used lowered from 50 ng to 20 ng.

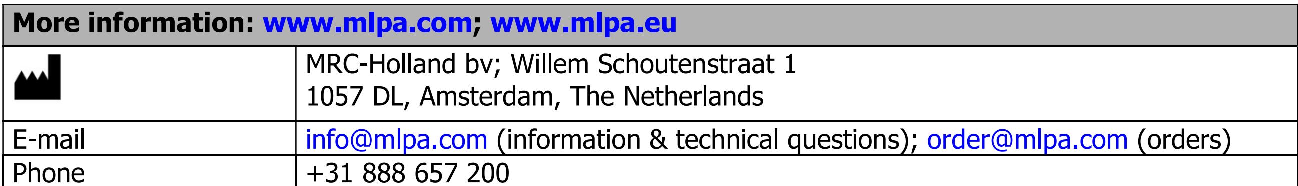
Version 21 – 27 November 2014
- Product description completely rewritten.

Version 20 – 09 November 2014 (53)
- Product description adapted to a new product lot (lot number added, new pictures included).

Version 19 – 26 November 2013 (52)
- Cell lysate preparation protocol removed.
- Note about data analysis software added.
- Recommendation of cell lysates as preferred sample type removed.

Version 18 – 18 November 2013 (50)
- Page 1 rewritten. Comment added about use of this product and recommendation to use QF-PCR.
- Reference list updated.
- Note on page 5 updated on the use of purified DNA and when to take the amniotic fluid sample.

Version 17 – 02 February 2013 (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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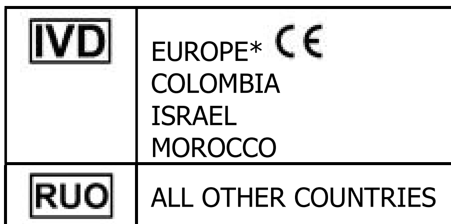

IVD	EUROPE* CE COLOMBIA ISRAEL MOROCCO
RUO	ALL OTHER COUNTRIES

*comprising EU (candidate) member states and members of the European Free Trade Association (EFTA). The product is for RUO in all other European countries.