Instructions for Use

SALSA® MLPA® Probemix P095 Aneuploidy

See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P095 Aneuploidy product page on our website to find Certificates of Analysis and a list of related products.

Product Name	SALSA® MLPA® Probemix P095 Aneuploidy
Version	A4
Catalogue numbers	P095-025R (25 reactions) P095-050R (50 reactions) P095-100R (100 reactions)
Basic UDI-DI	872021148P09569
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA ISRAEL MOROCCO
RUO	ALL OTHER COUNTRIES

Additional Test Components	Catalogue Numbers
SALSA® MLPA® Reagent Kit	EK1-FAM EK1-CY5 EK5-FAM EK5-CY5 EK20-FAM

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

Storage and Shelf Life

Recommended conditions		

A shelf life of until the expiry date is guaranteed, also after opening when stored in the original packaging under recommended conditions. For the exact expiry date, see the label on the vial. This product should not be exposed to more than 25 freeze-thaw cycles. Do not use the product if the packaging is damaged or opened. Leave chemicals in original containers. Waste material must be disposed of in accordance with the national and local regulations.

More Information: www.mrcholland.com	
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Any serious incident that has occurred in relation to this product should be reported to MRC Holland and the competent authority of the Member State or country in which the user and/or the patient is located.

Changes in this Product Version

As compared to version A3, the first nucleotide of the LPO was adjusted in three probes. No change in length or in the sequence detected by the probes. Version A4 is made suitable for use with a DNA input amount between 20-250 ng.

1. Intended Purpose

The SALSA MLPA Probemix P095 Aneuploidy is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications 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. For postnatal samples, genomic DNA isolated from human peripheral whole blood specimens can be used. For prenatal samples, genomic DNA isolated from (un)cultured amniotic fluid obtained in week 16 of the pregnancy or later and free from blood contamination, (un)cultured chorionic villi free from maternal contamination, or fetal blood can be used. P095 Aneuploidy is intended to confirm a potential cause for and clinical diagnosis of Patau, Edwards and Down syndromes (trisomy 13, 18 and 21, respectively) and Turner, Triple X, Klinefelter, and XYY syndromes (X/Y chromosome aneuploidies).

Assay results are intended to be used in conjunction with other clinical and diagnostic findings, consistent with professional standards of practice, including confirmation by alternative methods, clinical genetic evaluation, and counselling, as appropriate. The results of this test should be interpreted by a clinical molecular geneticist or equivalent.

This device is not intended to be used for standalone diagnostic purposes, pre-implantation testing, population screening, or for the detection of, or screening for, acquired or somatic genetic aberrations.

[1] Please note that this probemix is for IVD use in the countries specified on page 1 of this product description. In all other countries, this is a RUO product.

[2] To be used in combination with a SALSA MLPA Reagent Kit and Coffalyser.Net analysis software.

2. Sample Requirements

Specimen	20-250 ng purified human genomic DNA, free from heparin, dissolved in 5 µl $TE_{0.1}$ buffer, pH 8.0-8.5
Collection Method	Standard methods
Extraction Method	Methods tested by MRC Holland: • QIAGEN Autopure LS (automated) and QIAamp DNA mini/midi/maxi kit (manual) • Promega Wizard Genomic DNA Purification Kit (manual) • Salting out (manual)

Sample Types		
Test Sample	• Provided by user	
Reference Samples (Required)	• Provided by user • Extraction method, tissue type, DNA concentration and treatment as similar as possible in all test and reference samples. • Have a normal copy number and ≤0.10 standard deviation for all probes. • At least three* independent reference samples required in each experiment for proper data normalisation. Derived from unrelated **male** individuals from families without a history of developmental delay or congenital abnormalities.	
No-DNA Control (Preferably)	• Provided by user • $TE_{0.1}$ buffer instead of DNA • To check for DNA contamination	
Positive Control Samples (Preferably)	• Provided by user, or	
	Available from third parties	See the table of positive samples on the probemix product page on our website.
Validation Samples (Required)	• In the validation experiments of this probemix, DNA samples from healthy **male** individuals should be used.	

*When testing >21 samples, include one extra reference for each 7 test samples.

3. Test Procedure

See the MLPA General Protocol.

4. Quality Control, Data Analysis, and Troubleshooting

Quality Control Fragments in the Probemix	
Length (nt)	**Function**
64-70-76-82	DNA quantity control fragments
88-96	DNA denaturation control fragments
92	Benchmark fragment
100	Chromosome X presence control fragment
105	Chromosome Y presence control fragment

Coffalyser.Net should be used for data analysis in combination with the appropriate product and lot-specific Coffalyser sheet. See the Coffalyser.Net Reference Manual for details on data analysis and quality control.

For troubleshooting help, see the additional resources offered on our support portal.

5. Interpretation of Results

Determining Typical Values in Unaffected and Affected Populations
The typical final ratio (FR) values stated in the copy number tables below were determined in a validation study with samples containing abnormal copy numbers, and from Van Opstal et al. (2009). The standard deviation of each individual probe over all the reference samples was ≤0.10.

Reference Probes:
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 neutralises 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 FR values for various genotypes as determined by Coffalyser.Net software can be found in Table 1 below.

Table 1: Typical FRs in Unaffected and Affected Populations When Using 46,XY as Reference Samples

Sample type		Expected FR^				
		Chr. 13	Chr. 18	Chr. 21	Chr. X	Chr. Y
46,XY	**Male**	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20
46,XX	**Female**	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	1.65 - 2.25	0
47,XY,+13	Patau syndrome *	≥1.20 and ≥1.30*	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20
47,XY,+18	Edwards syndrome *	0.80 - 1.20	≥1.20 and ≥1.30*	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20
47,XY,+21	Down syndrome *	0.80 - 1.20	0.80 - 1.20	≥1.20 and ≥1.30*	0.80 - 1.20	0.80 - 1.20
45,X0	Turner syndrome	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0
47,XXX	Triple X syndrome	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	2.50 - 3.00	0
47,XXY	Klinefelter syndrome	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	1.65 - 2.25	0.80 - 1.20
47,XYY	XYY syndrome	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	0.80 - 1.20	1.65 - 2.25

^ All other values are ambiguous.
* A trisomy for a certain chromosome should be considered when the following two criteria are met:
I. At least 4 out of 8 FRs for a certain chromosome are ≥ 1.30 AND
II. At least 6 out of 8 FRs for that chromosome are ≥ 1.20.

Triploidies
This probemix may only be used to detect triploidies for RUO applications. The expected final FR values for triploidies as determined by Coffalyser.Net software are detailed in Table 2, and FRs are expected to centre around these values. **Importantly, MLPA cannot distinguish between 69,XXX and 46,XX samples.**

Table 2: Typical FRs of Triploidies When Using 46,XY as Reference Samples (for RUO)

Sample type		Expected FR				
		Chr. 13	Chr. 18	Chr. 21	Chr. X	Chr. Y
69,XXX *	Triploid female	1	1	1	2	0
69,XXY	Triploid male	1	1	1	1.33	0.67
69,XYY	Triploid male	1	1	1	0.67	1.33

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

6. Performance Characteristics

<table>
<tr><th>Study</th><th>Description</th></tr>
<tr><td>Expected values for copy number in normal and affected populations</td><td>To determine the expected values in normal and affected populations, a study was conducted on over 1,500 MLPA reactions using samples with and without abnormal copy numbers. Cut-off values for copy number determination were verified with P095 Aneuploidy in 44 postnatal cell line- and blood-derived DNA samples and two placenta DNA samples from healthy individuals with normal copy numbers, and 11 DNA samples with known aneuploidies. The aneuploid samples tested included individuals with Edward, Down, Patau, Turner, Triple X, Klinefelter and XYY syndromes. The expected FRs for the corresponding copy number were found in >99.8% (2,336/2,340) measurements.</td></tr>
<tr><td>Limit of Detection</td><td>A study using representative probemixes was conducted to evaluate the minimum and maximum amount of DNA acceptable as the assay input. Results support the use of 50-250 ng of human DNA as the recommend input amount. An additional validation study was conducted using the P095 Aneuploidy probemix to assess reduced DNA input amounts, and this demonstrated that reliable results can be obtained with 20 ng of DNA. These lower and higher limits of detection were further verified using P095 Aneuploidy on six postnatal cell line-derived DNA samples with known aneuploidies and one blood-derived DNA sample with normal copy number and expected results were obtained in >99% (1,004/1,008) of measurements using both the lower and upper input amount of DNA.</td></tr>
<tr><td>Interfering substances</td><td>A study using P095 Aneuploidy was performed to assess the potential for interference of endogenous and exogenous substances on six postnatal cell line-derived DNA samples with known aneuploidies and on one blood-derived DNA sample without any mutation. Tested potential interferents, their potential source, testing concentrations and results are presented in the table below.

FRs within the expected cut-off category were obtained for all samples in the presence of bilirubin. For EDTA, Fe^{3+} and heparin, >99% of measurements fell in the expected range, with the remaining measurements falling in the ambiguous range. For NaCl, >95% of measurements fell in the expected range, indicating that this had a moderate impact on probe performance. A small number of measurements fell in the incorrect range, however for these samples Coffalyser.Net issued warnings, which would require the assay to be repeated.

Hemoglobin had a more significant effect on measurements, where both ambiguous and incorrect results were observed for 22.2% of measurements. Importantly, Coffalyser.Net issued warnings for all hemoglobin-affected samples, which would also require the assay to be repeated.

<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>Bilirubin</td><td>Endogenous – fetal blood</td><td>1.5 mM</td><td>Copy number: Expected FR for 756/756 probes</td></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Copy number: Expected FR for 751/756 probes</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 725/756 probes</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Copy number: Expected FR for 755/756 probes</td></tr>
<tr><td>Heparin^</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 717/720 probes</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 588/756 probes</td></tr>
</table>
* Results are summarised for all probes across all six samples tested

^ One of the triplicates for one sample tested with this interferent also failed analysis in Coffalyser.Net, indicating poor quality.</td></tr>
<tr><td>Cross-reactivity</td><td>Cross-reactivity is the potential for probes to bind to homologous regions (e.g. pseudogenes) or other cross-reactive sequences. Quality tests were carried out on aneuploidy positive and wildtype cell line-, blood-derived, and placenta-derived DNA samples to determine whether probes are specific to their target sequence, and >99.8% (2,336/2,340) of measurements met the quality criteria for specificity.</td></tr>
<tr><td>Accuracy</td><td>Results of accuracy are derived from trueness and precision studies. For trueness, 12 previously genotyped cell line- and blood-derived DNA samples were tested using P095 Aneuploidy and found to have the expected results in >99.5% (681/684) of measurements.

Assay precision was tested by repeatedly testing samples with known copy number over multiple days, and by multiple operators. Results showed a correct call in 3,760/3,780 data points, leading to a precision of >99.4%.</td></tr>
<tr><td>Clinical validity*</td><td>The most common cases of an abnormal chromosome copy number at birth concern the presence of an extra chromosome 13, 18 or 21 (Patau, Edwards, or Down syndromes, respectively), each resulting in intellectual disability and other syndromic congenital abnormalities. Also common is the presence of an extra or missing sex chromosome (Turner, Triple X, Klinefelter, and XYY syndromes), which have a much less severe phenotypes than the aforementioned trisomies. For all of these syndromes, full aneuploidy accounts for the majority of cases, while a minority results from mosaicism or partial chromosome gain</td></tr>
</table>

Study	Description
	or loss. In addition, Patau and Down syndrome can occasionally result from unbalanced Robertsonian translocation. *Based on a 2000-2026 literature review

Summary of Safety and Performance (SSP)
The SSP is available in the European database on medical devices (Eudamed), https://ec.europa.eu/tools/eudamed, or upon request.

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Distance to next probe	Length (nt)	Probe number	Warnings
Xp22.12	4.8 **M**b	364	02906-L02300	
Xp21.3	6.2 **M**b	229	02898-L02369	«
Xp21.2	7.3 **M**b	454	01388-L28680	
Xp11.4	28.3 **M**b	274	02904-L02370	
Xq12	42.1 **M**b	154	00652-L00637	
Xq22.3	20.3 **M**b	184	02155-L01607	
Xq25	23.7 **M**b	409	00820-L28679	
Xq28		319	00819-L28681	
Yp11.31	0.4 kb	193	02152-L00592	
Yp11.31	173.8 kb	160	02153-L00596	
Yp11.31	11.2 **M**b	283	04189-L03982	
Yq11.21		240	01071-L00464	
13q13.1	4.1 **M**b	355	00452-L00360	
13q13.3	12.0 **M**b	178	00799-L00317	
13q14.2	1.6 **M**b	220	00582-L00147	
13q14.3	21.6 **M**b	400	00801-L00639	
13q21.33	23.4 **M**b	265	02125-L01636	
13q32.1	15.6 **M**b	148	00798-L00316	
13q34	586.3 kb	443	00802-L00320	«
13q34		310	00871-L00461	
18p11.32	13.2 **M**b	301	00811-L00329	
18p11.21	8.0 **M**b	436	02847-L02277	
18q11.2	18.3 kb	391	02846-L02276	
18q11.2	24.9 **M**b	211	02845-L02275	
18q21.2	8.9 **M**b	142	02127-L01638	
18q21.32	4.0 **M**b	172	00808-L00326	
18q21.33	15.6 **M**b	346	00810-L00328	
18q23		255	00807-L00325	
21q11.2	1.5 **M**b	247	00816-L00334	
21q21.1	5.6 **M**b	202	02115-L01605	
21q21.1	4.5 **M**b	166	00813-L00636	
21q21.3	5.2 **M**b	337	00812-L00330	
21q22.11	536.4 kb	427	00817-L00335	
21q22.11	5.1 **M**b	291	02116-L01604	
21q22.13	5.6 **M**b	136	00815-L00333	«
21q22.3		382	02834-L02265	

Probe lengths may vary slightly depending on capillary electrophoresis instrument settings. Please see the most up to date Coffalyser sheet for exact probe lengths obtained at MRC Holland. All autosomal probes (highlighted in grey) are set as both target and reference probes. For further details, see Section 5: Interpretation of Results. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

« These probes are 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.

Probemix-specific precautions

1. This product is not known to contain any harmful agents. Based on the concentrations present, none of the ingredients are hazardous as defined by the Hazard Communication Standard. **A Safety Data Sheet (SDS) is not required for this product**: none of the ingredients contain dangerous substances at concentrations requiring distribution of an SDS (as per Regulation (EC) No 1272/2008 [EU-GHS/CLP] and 1907/2006 [REACH] and amendments).
2. Sample or technical artefacts may appear as a (mosaic) copy number abnormality affecting one or more target regions. Whole/partial chromosome copy number changes should therefore be confirmed by analysis of an independent DNA sample, to exclude false positive results.
3. Small changes (e.g. SNVs, small indels) in the sequence targeted by a probe can cause false positive results, even when >20 nt from the probe ligation site. Sequence changes 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. Deviations detected by this product should be confirmed, and single-probe deviations always require confirmation. Sequencing of the target region is recommended. Please contact MRC Holland for more information: info@mrcholland.com.
4. This probemix is validated for use with 20-250 ng DNA input. Samples with a lower DNA input than 20 ng, e.g. blastomeres, cannot be used for analysis using this probemix.
5. DNA from whole genome amplification reactions (WGA) is not suitable for MLPA due to amplification bias.
6. P095 probe sequences have been selected based on their location on a specific chromosome, not on being located in a specific gene, we recommend disregarding copy number variations detected by a single probe. Copy number changes detected by only a single probe may be a false positive result due to, for example, a mutation or polymorphism that prevents ligation of the MLPA probe, or that destabilises the binding of a probe oligonucleotide to the sample DNA.

7. All autosomal probes detecting loci on chromosomes 13, 18, and 21 are set as reference probes. Accordingly, in full trisomy cases, the values for all eight reference probes detecting the same chromosome will therefore be increased. This can result in a Reference Probe Quality (RPQ) warning in Coffalyser.Net. This is acceptable providing that other quality scores do not have warnings.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. This probemix can only be used to detect triploidies in a research setting and not for diagnostic use. MLPA cannot distinguish between diploid females (46,XX) and triploid females (69,XXX).
2. When using on (un)cultured amniocytes and (un)cultured chorionic villi, contamination of the sample with maternal DNA may lead to wrong conclusions. Contamination of fetal DNA samples with maternal DNA can be detected by QF-PCR, but not by MLPA.
3. MLPA cannot discriminate between maternal and fetal DNA in a sample, therefore, it is not possible to use maternal blood samples for prenatal diagnosis.
4. When using on (un)cultured chorionic villi, discrepancies in chromosomal patterns between DNA from chorionic villi and fetus 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).
5. Mosaicism can occur in all syndromes detected by this probemix, and FRs are affected by the proportion of affected cells in the tissue sample from which the DNA was purified. The use of the fixed cut-off values as mentioned in the table under section 5 will not allow the detection of aneuploidies in all samples that are a mixture of normal and abnormal cells, such as mosaic samples. In case several probes targeting adjacent sequences have an unusual value, but do not reach the usual threshold values for a aneuploidy, mosaicism is a possible cause. 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. Mosaic mutations may not always be detectable with MLPA.
6. A minor proportion cases of Patau, Edwards and Down syndrome cases can be caused by a partial chromosome gain. If a partial chromosome gain has occurred, a duplication of a specific region will result in a FR > 1.30 of two or more of the corresponding probes. Additional testing should be used to confirm and interpret the result.

9. References Cited in this IFU

1. Van den Berg C et al. (2006). (Potential) false-negative diagnoses in chorionic villi and a review of the literature. Prenat Diagn. 26:401-408.
2. Van Opstal D et al. (2009). Rapid aneuploidy detection with multiplex ligation-dependent probe amplification: a prospective study of 4000 amniotic fluid samples. Eur J Hum Genet. 17:112-121.

Implemented changes in the product description
Version A4-07 – 28 August 2026 (03S) - Basic UDI-DI added. - Performance characteristics table added. - Reference to SSP added. - Intended purpose updated to add 'manual' to the assay type, remove buccal swabs as a sample type, edit the syndrome name from '47,XYY syndrome' to 'XYY syndrome', and to remove that the probemix cannot distinguish between normal females (46,XX) and triploid females (69,XXX). - Interpretation of Results section rewritten. Written guidance for expected FR ranges converted to table format. Expected FRs for triploid samples included in separate table for research use only. - Salt sensitivity probe warnings removed for X chromosome probes 00652-L00637 and 00819-L28681. - Precaution added that RPQ warning may results in full trisomy cases. - Limitation added that the probemix can only be used to detect triploidies in a research setting. - Limitation added regarding mosaicism. - Limitation added regarding partial chromosome gains. - Various minor textual changes throughout document. - Probemix is now IVDR certified.

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