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.

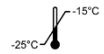
Product Name	**SALSA® MLPA® Probemix P095 Aneuploidy**
Version	A4
Catalogue numbers	P095-025R (25 reactions) P095-050R (50 reactions) P095-100R (100 reactions)
Basic UDI-DI	n.a.
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 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 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 or buccal swabs 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, 47,XYY syndromes (X/Y chromosome aneuploidies). This probemix cannot distinguish between normal females (46,XX) and triploid females (69,XXX).

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 or 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 Normal and Affected Populations

The typical final ratio (FR) values stated in the copy number tables were determined in a validation study with samples containing abnormal copy numbers. 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 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 final ratio (FR)-values for various genotypes as determined by Coffalyser.Net software can be found in the table below.

Typical Results of Aneuploidy when using 46,XY as reference samples.

Sample type		Expected FR				
		Chr. 13	**Chr. 18**	**Chr. 21**	**Chr. X**	**Chr. Y**
46,XY	**Male**	**1**	**1**	**1**	**1**	**1**
46,XX *	**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 *	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.

The table illustrates the relationship between final ratio and corresponding chromosomal aneuploidy. Test results are expected to center around these values. Ambiguous values can indicate a technical problem, but may also reflect a biological cause such as mosaicism or a SNV influencing a single probe. It is important to use Coffalyser.Net to determine the significance of values found.

For copy number changes of a complete chromosome (majority of cases):

In theory, all probes targeting a certain chromosome should give approximately the same FR value, also in cases of aneuploidy. In practice, however, certain variables such as SNVs, 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 FR > 1.30 of two or more of the corresponding probes. Additional testing should be used to confirm and interpret the result.
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 has low variability.

6. Performance Characteristics

Patau, Edwards and Down syndromes are caused and defined by the presence of trisomy 13, 18 and 21, respectively. The diagnosis for these three trisomies, as well as the diagnosis of Turner, Triple X, Klinefelter and other sex chromosome syndromes, is dependent on the presence of the corresponding aneuploidy. There are no other (non)genetic causes for these syndromes, except for some rare cases of partial aneuploidy and mosaicism.

Several studies have reported a diagnostic and analytical sensitivity and specificity >99% for the detection of chromosomes 13, 18, 21, X and Y aneuploidies using SALSA MLPA probemix P095 Aneuploidy, with a 100% accuracy for the detection of full trisomies 13, 18 and 21 (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: SNVs or other polymorphisms 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.

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 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 change of the whole/partial gene. Whole/partial gene deletions or duplications 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. DNA amplification methods cannot be used due to amplification bias.
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 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.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. MLPA cannot distinguish between normal females (46,XX) and triploid females (69,XXX).
2. For use 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. For use 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).

9. References Cited in this IFU

1. Boormans EM et al. (2010). Comparison of multiplex ligation-dependent probe amplification and karyotyping in prenatal diagnosis. Obstet Gynecol. 115:297-303.
2. Gerdes T et al. (2008). Multiplex ligation-dependent probe amplification (MLPA) in prenatal diagnosisexperience of a large series of rapid testing for aneuploidy of chromosomes 13, 18, 21, X and Y. Prenat Diagn. 28:1119-1125.
3. Kooper AJ et al. (2008). Multiplex ligation-dependent probe amplification (MLPA) as a stand-alone test for rapid aneuploidy detection in amniotic fluid cells. Prenat Diagn. 28:1004-1010.
4. 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.
5. 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 A4-06 – 4 April 2025 (03S) - Product description rewritten and adapted to a new template.