Instructions for Use
SALSA® MLPA® Probemix P155 EDS

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 P155 EDS product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P155 EDS**
Version	E1
Catalogue numbers	P155-025R (25 reactions) P155-050R (50 reactions) P155-100R (100 reactions)
Basic UDI-DI	872021148P15562
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

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

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.

Regulatory Status	
IVD	EUROPE CE 2797
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

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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 compare to version D2, ten new target probes have been included and one flanking probe has been removed. Five reference probes have been replaced and one reference probe removed. Lengths of ten probes have been adjusted.

1. Intended Purpose

The SALSA MLPA probemix P155 EDS is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *COL3A1* and *TNXB* genes in genomic DNA isolated from human peripheral blood specimens in order to confirm a potential cause for and clinical diagnosis of vascular Ehlers-Danlos syndrome (vEDS) (in the context of a monoallelic pathogenic variant in *COL3A1*) and classical-like Ehlers-Danlos syndrome (clEDS) (in the context of biallelic pathogenic variants in *TNXB*), respectively. This product can also be used for molecular genetic testing of at-risk family members[3].

Copy number variations (CNVs) detected with P155 EDS should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. Most defects in the *COL3A1* and *TNXB* genes are point mutations, none of which will be detected by MLPA. It is therefore recommended to use this assay in combination with sequence analysis.

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 or prenatal 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.

[3] Certain probes targeting additional genes included in P155 EDS may only be used in a research setting. The following table summarises which probes are for IVD use or exclusively restricted to be used in a research setting:

IVD Targets	RUO Targets
CO3A1, TNXB	*CYP21A2, ATF6B*

2. Sample Requirements

Specimen	50-250 ng purified human genomic DNA, 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 individuals from families without a history of clEDS or vEDS.	
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)	• This probemix contains target probes that target sequences with natural variation. The validation experiments of this probemix should result in a standard deviation ≤0.10 for all reference probes.	

*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.

Expected Results of Reference Probes

Final Ratio (FR)	Copy Number	Description
0.80 – 1.20	2	Normal

Typical Results of Probes Targeting Two Copies (*COL3A1*/*TNXB*/flanking probes)

Final Ratio (FR)	Copy Number	Description
0	0	Homozygous deletion
0.40 – 0.65	1	Heterozygous deletion
0.80 – 1.20	**2**	**Normal**
1.30 – 1.65	3	Heterozygous duplication
1.75 – 2.15	4	Homozygous duplication or Heterozygous triplication
All other values	-	Ambiguous

The tables illustrate the relationship between final probe ratio and corresponding copy number. 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.

6. Performance Characteristics

Study	Description
Expected values for copy number in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1500 MLPA reactions using samples with and without abnormal copy numbers. Cut-off values for copy number determination were verified with P155 EDS in 23 blood-derived samples from healthy (mainly European individuals) as well as 41 commercially available samples with no reported data concerning CNVs in the targeted region and four samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested.
Limit of detection	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. These lower and higher limits of detection were verified using P155 EDS on two samples with known CNV status and on one sample without any mutation and expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	A study using P155 EDS was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNV status. For most probes, expected FRs (FRs within the expected cut-off category) were obtained even in the presence of potential interferents at concentrations shown in the table below.

Interferent	Source	Testing Concentration	Results*
EDTA	Exogenous – specimen collection tubes	1.5 mM	Copy number: Expected FR for 378/378 measurements
NaCl	Exogenous – DNA extraction	40 mM	Copy number: Expected FR for 377/378 measurements
Fe^{3+} ($FeCl_3$)	Exogenous – DNA extraction	1 µM	Copy number: Expected FR for 375/378 measurements
Heparin	Exogenous – specimen collection tubes	0.02 U/mL	Copy number: Expected FR for 378/378 measurements
Hemoglobin	Endogenous – blood sample	0.02 µg/µl	Copy number: Expected FR for 267/378 measurements

* Results are summarised for all probes across all three samples tested.

Hemoglobin had the largest effect on copy number determination: FRs within an incorrect range were obtained in all samples. DNA extraction methods from blood remove hemoglobin and during testing of 23 samples extracted from blood the expected FRs were found. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found.

Study	Description
	NaCl and Fe^{3+} had milder effects on copy number determination: all final ratios outside the expected range were ambiguous range. Ambiguous values would not lead to false positives or false negatives. At most, these would lead to a retest. Coffalyser.Net issues warnings for the samples in which the interferents showed an effect, as well as lowered quality scores.
Cross-reactivity	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 to determine whether probes are specific to their target sequence and all probes met the quality criteria for specificity.
Accuracy	Results of accuracy are derived from trueness and precision studies. For trueness, five previously genotyped samples were tested using P155 EDS and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number status over multiple days, and by multiple operators. Results showed a correct call in 1889/1890 data points, leading to a precision of 99%.
Clinical validity*	*COL3A1*: ~1% of vEDS is caused by deletions in *COL3A1* (Gene Reviews)[1]. *TNXB*: ~10% of clEDS is caused by deletions involving *TNXB* (Gene Reviews)[2]. *Based on a 2010-2026 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.

[1] Gene Reviews – Vascular Ehlers-Danlos Syndrome
[2] Gene Reviews – TNXB-Related Classical-Like Ehlers-Danlos Syndrome

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Exon	Distance to next probe	Mutation	Length (nt)	Probe number	Warnings
2q32.2	*COL3A1*	Exon 1	0.1 kb		196	05019-L04405	
2q32.2	*COL3A1*	Exon 1	10.3 kb		148	05018-L04404	
2q32.2	*COL3A1*	Exon 2	0.9 kb		221	05020-L21329	
2q32.2	*COL3A1*	Exon 4	1.4 kb		372	17522-L21421	
2q32.2	*COL3A1*	Exon 5	1.5 kb		178	05021-L04407	
2q32.2	*COL3A1*	Exon 7	1.5 kb		471	22231-L31336	
2q32.2	*COL3A1*	Exon 9	0.9 kb		319	17521-L21420	
2q32.2	*COL3A1*	Exon 11	1.2 kb		274	05022-L21402	
2q32.2	*COL3A1*	Exon 14	1.2 kb		161	17517-L21416	
2q32.2	*COL3A1*	Exon 16	0.7 kb		250	22230-L31335	
2q32.2	*COL3A1*	Exon 17	0.7 kb		400	05023-L04409	
2q32.2	*COL3A1*	Exon 20	0.8 kb		364	05024-L04410	
2q32.2	*COL3A1*	Exon 22	0.5 kb		265	22229-L31334	+
2q32.2	*COL3A1*	Exon 23	1.6 kb		136	17516-L21415	
2q32.2	*COL3A1*	Exon 27	0.6 kb		288	22228-L31333	
2q32.2	*COL3A1*	Exon 28	1.6 kb		427	17523-L21422	
2q32.2	*COL3A1*	Exon 32	1.7 kb		452	05026-L23956	
2q32.2	*COL3A1*	Exon 34	1.4 kb		350	22227-L31332	
2q32.2	*COL3A1*	Exon 36	1.2 kb		260	20545-L31302	
2q32.2	*COL3A1*	Exon 39	1.2 kb		494	22360-L31519	
2q32.2	*COL3A1*	Exon 41	1.1 kb		131	22225-L31330	
2q32.2	*COL3A1*	Exon 43	1.1 kb		256	05223-L31492	
2q32.2	*COL3A1*	Exon 45	0.6 kb		409	22224-L31329	
2q32.2	*COL3A1*	Exon 47	2.2 kb		226	20544-L28408	
2q32.2	*COL3A1*	Exon 49	1.5 kb		333	22223-L31895	
2q32.2	*COL3A1*	Exon 51			486	05028-L31311	
6p21.32	*CYP21A2*	Exon 3	0.7 kb	c.332_339del	185	15221-L20260	∞ ¬ ๑
6p21.32	*CYP21A2*	Exon 6	4.0 kb	V237E	232	15216-L16990	∞ ¬ # ๑
6p21.32	*TNXB*	Exon 35	<0.1 kb		477	13292-L31309	ท ๑
6p21.32	*TNXB*	Exon 35	2.6 kb		154	13291-L14636	ท ๑
6p21.32	*TNXB*	Exon 31	6.3 kb		296	01982-L31303	
6p21.32	*TNXB*	Exon 26	4.1 kb		418	05016-L15002	#
6p21.32	*TNXB*	Exon 23	8.3 kb		391	05015-L04401	± #
6p21.32	*TNXB*	Exon 19	3.8 kb		461	05014-L31308	
6p21.32	*TNXB*	Exon 16	0.8 kb		436	17524-L21423	
6p21.32	*TNXB*	Exon 15	0.6 kb		382	05013-L14827	
6p21.32	*TNXB*	Exon 14	3.6 kb		310	03033-L31304	#
6p21.32	*TNXB*	Exon 12	5.2 kb		208	05012-L31728	
6p21.32	*TNXB*	Exon 11	5.5 kb		283	05011-L04397	∫
6p21.32	*TNXB*	Exon 8	4.7 kb		238	05010-L04396	
6p21.32	*TNXB*	Exon 5	7.4 kb		190	05009-L22188	
6p21.32	*TNXB*	Exon 3	1.1 kb		172	05008-L15001	
6p21.32	*TNXB*	Exon 2	11.4 kb		215	17518-L21417	
6p21.32	*TNXB*	Exon 1	0.3 kb		326	01980-L21389	
6p21.32	*TNXB*	Upstream (Exon 1)	18.6 kb		142	05007-L04393	Ø
6p21.32	*ATF6B*				340	01979-L31305	¬
2p	Reference				504	09870-L19465	
5q	Reference				124	18709-L21056	
6q	Reference				244	21641-L30683	
7q	Reference				166	05721-L31493	
11q	Reference				202	08187-L08081	
17q	Reference				303	15461-L17667	
18q	Reference				358	16442-L18895	
19p	Reference				444	09077-L23425	

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.
The *COL3A1, TNXB, and CYP21A2* exon numbers are derived from MANE project and are based on MANE Select transcript. For more information, see the probe sequences document available on the product page at www.mrcholland.com. Annotation of one probe with a target at the edge of or slightly outside the coding region was altered. The exon numbering from the previous version of this product description is disclosed between brackets.
Chromosomal bands are based on: hg18.

7. Precautions and Warnings

Probe warnings

∞	Wild type sequence detected. These probes detect *CYP21A2*-specific sequences. The 185 nt probe signal is decreased in the presence of the 8 bp deletion *CYP21A2* c.332_339del. The 232 nt probe signal is decreased in the presence of the *CYP21A2* V237E mutation and to a lesser extent in the presence of the *CYP21A2* M240K mutation. Due to the high homology of *CYP21A2* with the *CYP21A1P* pseudogene, gene conversions can occur. Gene conversions can be detected as apparent deletions or duplications.
¬	These probes are flanking probes, included to help determine the extent of a deletion/duplication. Copy number alterations of flanking probes are unlikely to be related to the condition tested.
±	The presence of single nucleotide variant (SNV) rs369180703 may be due to a micro gene conversion between *TNXB* and a homologous target and can affect the signal of this probe.
Ø	This probe targets a sequence outside of the known coding region. Copy number alterations of only this probe are of unknown clinical significance.
#	The specificity of these probes relies on a single nucleotide difference compared to a related gene or pseudogene. As a result, an apparent duplication of only these probes can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
+	The ligation site of this probe is >20 nt away from the nearest exon. For more information, download the probe sequences document available on the product page at www.mrcholland.com.
∫	For this 283 nt probe, it has been experimentally confirmed that a single nucleotide change in a related sequence can result in a false duplication result; rs61740331 in *TNXB* exon 28 creates a second genomic target resulting in an increased final ratio. In case of an apparent duplication of only this probe, it is recommended to sequence the genomic region surrounding rs61740331.
и	These probes are within the 120 bp sequence that is absent in the *TNXA* pseudogene and can therefore be used to detect the frequent 120 bp deletion *TNXB* c.11435_11524+30 deletion (exon 35).
⍟	CNVs detected with these probes are frequent in the general population.

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. SNVs rs369180703 and rs61740331 mentioned in this document require additional caution. Please note that any probe can be affected by known or novel SNVs.
5. Copy number alterations of reference probes are unlikely to be related to the condition tested.
6. Before testing patient samples, testing of samples from healthy individuals is required to identify suitable reference samples for proper data analysis.

Technique-specific precautions

See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. Due to the high homology between *TNXB* exons 32-34 and 36-44 and the corresponding exons in its pseudogene *TNXA*, no probes can be designed that are specific for only these *TNXB* exons.
2. clEDS is caused by biallelic pathogenic variants in *TNXB*. In cases where heterozygous deletions are identified with P155 EDS, additional molecular techniques should be used to investigate compound heterozygous variants.
3. Biallelic pathogenic variants of *CYP21A2* are associated with Congenital Adrenal Hyperplasia (CAH). Approximately 10% of CAH patients also have *TNXB* deficiency. This contiguous gene syndrome is referred to as CAH-X, and is the result of a non-functional *TNXB-TNXA* chimeric fusion gene causing a deletion of *CYP21A2*, which is located between *TNXA* and *TNXB*. Previously described CAH-X CH-1 *TNXA-TNXB* fusions (presenting as the 120 bp *TNXB* deletion) can be detected by P155 EDS (Burch et al. 1997, Morissette et al. 2015). To determine the copy number of *CYP21A2* and to detect *CYP21A1P-CYP21A2* fusion genes in patients suspected of CAH, however, SALSA MLPA Probemix P050 CAH should be used. In P155 EDS, probes for *CYP21A2* and *ATF6B* are only included to help determine the extent of a *TNXB* deletion/duplication and are not for diagnostic use.

Technique-specific limitations

See the MLPA General Protocol.

9. References Cited in this IFU

1. Burch GH et al. (1997). Tenascin-X deficiency is associated with Ehlers-Danlos syndrome. *Nat Genet.* 17:104-108.
2. Morissette R et al. (2015). Broadening the Spectrum of Ehlers Danlos Syndrome in Patients With Congenital Adrenal Hyperplasia. *J Clin Endocrinol Metab.* 100:E1143-1152.

Implemented changes in the product description
Version E1-05 – 28 August 2026 (03S) - Product description adapted to new template. - Intended purpose updated: assay specified to be manual, “classical”-like EDS used instead of “classic”; duplications of target genes no longer included; monoallelic and biallelic deletion types specified; sentence regarding CAH-X removed; footnote for RUO and IVD probes added. - Performance characteristics updated. - Reference to SSP added. - Basic UDI-DI added. - Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites. - Warning for a probe targeting a sequence outside of the known coding region added for probe 05007-L04393. - Warning for a probe with a ligation site >20nt from the nearest exon added for probe 22229-L31334. - A probemix-specific limitation regarding *TNXA-TNXB* and *CYP21A1P-CYP21A2* fusion genes added. - A probemix-specific limitation for the investigation of compound heterozygous *TNXB* variants added. - Probemix is now IVDR certified.

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