Instructions for Use
SALSA® MLPA® Probemix P250 DiGeorge

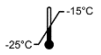
See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit, and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P250 DiGeorge product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P250 DiGeorge**
Version	B2
Catalogue numbers	P250-025R (25 reactions) P250-050R (50 reactions) P250-100R (100 reactions)
Basic UDI-DI	872021148P2505V
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 COLOMBIA ISRAEL
RUO	ALL OTHER COUNTRIES

Label Symbols			
IVD	In Vitro Diagnostic	RUO	Research Use Only

More Information: www.mrcholland.com	
	MRC Holland BV; Willem Schoutenstraat 1 1057 DL, Amsterdam, the Netherlands
E-mail	info@mrcholland.com (information & technical questions); order@mrcholland.com (orders)
Phone	+31 888 657 200

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 B1, six probes have been replaced.

1. Intended Purpose

The SALSA MLPA Probemix P250 DiGeorge is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions or duplications in the human 22q11 region in genomic DNA isolated from human peripheral whole blood specimens, (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. P250 DiGeorge is intended to confirm a potential cause for and clinical diagnosis of 22q11.2 deletion syndrome (including, among others, DiGeorge syndrome, velocardiofacial syndrome, and conotruncal anomaly face syndrome), 22q11.2 duplication syndrome, or cat eye syndrome (22q11 duplications), and for testing of at-risk family members. It further contains probes for the detection of deletions in the 8p23.1 and 10p14 regions for a differential diagnosis of 22q11.2 deletion syndrome[3].

Copy number variations (CNVs) detected with P250 DiGeorge should be confirmed with a different technique. In particular, CNVs detected by only a single probe always require confirmation by another method. 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, parental evaluation, 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, 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 regions included in P250 DiGeorge 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
22q11 region, 8p23.1 region, 10p14 region 10p12.31 region	4q35-qter region, 9q34.3 region, 17p13.3 region, 22q13.33 region

2. Sample Requirements

Specimen	50-250 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 (for peripheral blood only): • 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 22q11.2 deletion syndrome, 22q11.2 duplication syndrome, cat eye syndrome, 8p23 deletion syndrome, or 10p deletion syndrome (also referred to as DiGeorge syndrome/velocardiofacial syndrome complex 2, or hypoparathyroidism, sensorineural deafness and renal dysplasia).	
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.

*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.

Typical Results of Probes Targeting Two Copies

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

Probes targeting the 8p23.1, 10p14, 10p12.31, 4q35-qter, 9q34.3, 17p13.3, and 22q13.33 regions are also used as reference probes for sample normalisation. In cases where FRs indicative of a CNV in one of these regions is obtained, Coffalyser.Net issues an RPQ warning for variable reference probes. This warning is acceptable in such cases and data analysis can still take place.

The tables illustrate the relationship between final 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

<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 1500 MLPA reactions using samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the ranges stated in the copy number table in the product description can be used.

Cut-off values for copy number determination were verified with SALSA MLPA Probemix P250 DiGeorge in 47 samples from healthy individuals with normal copy number and 12 samples with known CNVs. The expected FRs for the corresponding copy number were found in most samples tested. Overall, 99% (2534/2546) correct calls were obtained over all tested samples.</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. The use of insufficient or too much sample DNA can affect performance.

These lower and higher limits of detection were verified using SALSA MLPA Probemix P250 DiGeorge on five samples with known CNV status and on one sample without any mutation and expected results were obtained in most cases using both the lower and upper input amount of DNA. Overall, 98% (896/912) correct calls were obtained.</td></tr>
<tr><td>Interfering substances</td><td>SNPs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl or KCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using SALSA MLPA Probemix P250 DiGeorge 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.
<table>
<tr><th>Interferent</th><th>Source</th><th>Testing Concentration</th><th>Results*</th></tr>
<tr><td>EDTA</td><td>Exogenous – specimen collection tubes</td><td>1.5 mM</td><td>Expected FR for 638/684 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Expected FR for 627/684 measurements</td></tr>
<tr><td>Fe^{3+} ($FeCl_3$)</td><td>Exogenous – DNA extraction</td><td>1 µM</td><td>Expected FR for 661/684 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Expected FR for 647/684 measurements</td></tr>
<tr><td>Haemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Expected FR for 155/684 measurements</td></tr>
</table>
* Results are summarised for all probes across all six samples tested.

Hemoglobin had the largest effect on copy number determination: FRs within an incorrect range were obtained in all samples. Importantly, warnings or errors were obtained in all affected samples using Coffalyser.Net software. Therefore, it is only when hemoglobin is in excess that deviating probe signals can be found.</td></tr>
</table>

Study	Description
	Moreover, NaCl led to false positive results being obtained in all samples. Excess salt in a sample can lead to denaturation issues. Probes in P250 DiGeorge which are affected by NaCl are indicated by a warning in the product description. Other than the above-mentioned deviations, EDTA and Fe^{3+} led to ambiguous measurements being obtained. To minimise variability across 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.
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, 12 previously genotyped samples were tested using SALSA MLPA Probemix P250 DiGeorge 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 3311/3420 data points, leading to a precision of 97%.
Clinical validity*	100% of 22q11.2 Deletion Syndrome is explained by deletions in 22q11.2, and 100% of 22q11.2 Duplication Syndrome is explained by duplications in 22q11.2, and 100% of Cat Eye Syndrome is explained by duplications or triplications of 22q11. 100% of 8p23 deletion syndrome, 10p deletion syndrome (also referred to as DiGeorge syndrome/velocardiofacial syndrome complex 2 or HDR) is caused by deletions in 8p23[1] and 10p14-10p12.31[2,3]. *(Based on a 2008-2025 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] Orphanet - 8p23.1 microdeletion syndrome
https://www.orpha.net/en/disease/detail/251071#:~:text=Disease%20definition,abnormalities%2C%20and%20congenital%20heart%20defects
[2] OMIM - Digeorge syndrome/velocardiofacial syndrome complex 2 https://omim.org/entry/601362
[3] Orphanet - Hypoparathyroidism-sensorineural deafness-renal disease syndrome https://www.orpha.net/en/disease/detail/2237

Content – Probe Details Sorted by Chromosomal Position

Chr. position	Target	Distance to next probe	Length (nt)	Probe number	Warnings
Cat eye syndrome (CES) region					
22q11.1	*IL17RA*	463.6 kb	335	01082-L15012	
22q11.21	*SLC25A18*	183.4 kb	142	05457-L07613	
22q11.21	*BID*	98.0 kb	357	01767-L07389	
22q11.21	*MICAL3*	308.3 kb	178	05458-L04861	
22q11.21	*USP18*	608.7 kb	226	07528-L04863	
End of CES region; Start DiGeorge syndrome (DGS) region; Probes in region LCR22A-LCR22B					
22q11.21	*CLTCL1*	77.4 kb	191	05462-L05809	∫
22q11.21	*HIRA*	148.5 kb	160	01214-L02328	∫
22q11.21	*CDC45*	43.8 kb	466	05463-L05808	∫
22q11.21	*CLDN5*	200.2 kb	196	01218-L06270	∫
22q11.21	*GP1BB*	35.6 kb	208	05464-L10114	« ∫
22q11.21	*TBX1*	6.2 kb	172	05408-L07614	« ∫ ±
22q11.21	*TBX1*	132.9 kb	245	10810-L14347	« ∫
22q11.21	*TXNRD2*	187.4 kb	238	01223-L05814	Ø ∫
22q11.21	*DGCR8*	625.8 kb	148	08475-L08486	∫
Probes in region LCR22B-LCR22C					
22q11.21	*ZNF74*	93.9 kb	202	05927-L07395	∫
22q11.21	*KLHL22*	93.4 kb	283	01227-L05815	∫
22q11.21	*MED15*	305.3 kb	316	01231-L15877	∫
Probes in region LCR22C-LCR22D					
22q11.21	*SNAP29*	107.2 kb	373	01235-L00773	∫
22q11.21	*LZTR1*	450.3 kb	418	01521-L00951	∫
End 22q11.2 DGS region; Probes in region LCR22D-LCR22E					
22q11.21	*HIC2*	250.2 kb	308	13302-L15009	
22q11.21	*PPIL2*	273.3 kb	129	07529-L04870	
22q11.22	*TOP3B*	1.1 **M**b	292	13299-L14649	~
Probes in region LCR22E-LCR22F					
22q11.22	*RSPH14*	61.3 kb	261	08484-L09139	«
22q11.22	*GNAZ*	17.1 kb	214	08478-L08489	
22q11.23	*RSPH14*	5.0 kb	454	08479-L08490	
22q11.23	*RAB36*	641.8 kb	342	05932-L04872	+
Probes in region LCR22F-LCR22G					
22q11.23	*SMARCB1*	47.0 kb	220	05928-L07969	
22q11.23	*SMARCB1*	777.3 kb	400	05933-L05812	
Probe in region LCR22G-LCR22H					
22q11.23	*SNRPD3*		166	08481-L08492	
Probes in regions resulting in overlapping 22q11.2 DS features: used for data normalisation					
4q35-qter region					
4q35.1	*SLC25A4*	1.1 **M**b	326	12093-L15011	
4q35.2	*KLKB1*		184	01217-L00694	
8p23.1 region					
8p23.1	*PPP1R3B*	1.1 **M**b	472	01243-L07392	
8p23.1	*MSRA*	1.5 **M**b	427	01240-L00787	
8p23.1	*GATA4*		274	01226-L03844	
9q34.3 region					
9q34.3	*EHMT1*	74.1 kb	136	05059-L07380	
9q34.3	*EHMT1*		154	05058-L07382	
10p14 and 10p12.31 region					
10p14	*GATA3*	15.2 kb	267	01225-L09140	
10p14	*GATA3*	2.4 **M**b	301	07636-L07321	
10p14	*CELF2* Region	428.1 kb	350	01232-L17068	Ø
10p14	*CELF2* Region	230.5 kb	364	01234-L00781	Ø
10p14	*CELF2*	10 **M**b	382	01522-L00952	
10p12.31	*NEBL*		487	08480-L15878	+
17p13.3 region					
17p13.3	*RPH3AL*	14.3 kb	255	01735-L07385	
17p13.3	*RPH3AL*	413.0 kb	434	04081-L25903	
17p13.3	*GEMIN4*	614.6 kb	409	01238-L07390	
17p13.3	*YWHAE*		391	13603-L03531	#
22q13.33 region					
22q13.33	*ARSA*	94.3 kb	445	01093-L00661	
22q13.33	*SHANK3*		232	06787-L07383	«

The name of the regions of low-copy number repeats (LCRs) is based on (Burnside 2015).
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.
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.
Ø	These probes target sequences outside of the known coding region. Copy number alterations of only one of these probes are of unknown clinical significance.
#	The specificity of this probe relies on a single nucleotide difference compared to a related gene or pseudogene. As a result. an apparent duplication of only this probe can be the result of a non-significant single nucleotide sequence change in the related gene or pseudogene.
+	The ligation site of these probe(s) 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.
∫	These probes are expected to have approximately 35-50% reduced peak height in most 22q11.2 deletion syndrome patients (LCR22A – LCR22D deletion).
±	The presence of single nucleotide variant (SNV) rs72646950 can affect the signal of this probe.
~	This probe has been reported to have deviating copy numbers in a small number of healthy individuals. An apparent deletion or duplication detected by only this probe is of unknown clinical significance.

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. SNV rs72646950 mentioned in this document requires additional caution. Please note that any probe can be affected by known or novel SNVs.
5. Cat eye syndrome is usually the result of a small supernumerary chromosome of the 22q11 CES region. **WARNING**: The extra chromosome present in Cat Eye Syndrome patients is easily lost during postzygotic divisions, resulting in mosaicism. Detection of cat eye syndrome in mosaic samples might be better done by standard karyotype analysis or by FISH (see below). MLPA measures the average copy number of the CES region and may not be able to detect cat eye syndrome in mosaic samples that contain predominantly normal cells.
6. Please note that not all abnormalities detected by the SALSA MLPA Probemix P250 DiGeorge can be confirmed by FISH in 2025: FISH probe D22S553 is located around the *CLTCL1* gene, FISH probe D22S609 is located between *CLTCL1* and *HIRA*, and FISH probe D22S942 is located between *HIRA* and *CDC45L*. The clinical consequences of CNVs which cannot be confirmed by FISH may require further investigation.
7. This P250 DiGeorge probemix contains 17 probes for relevant regions of disorders with phenotypic features of 22q11.2 deletion syndrome on chromosomes 4q, 8p, 9q, 10p, 17p. Furthermore, two probes on 22q13.33 are present. These 19 probes are also used as reference probes for sample normalisation. Please inform MRC Holland (info@mrcholland.com) when a deletion of one or more of the probes outside the 22q11 region is detected in a patient with a 22q11.2 deletion syndrome.
8. This document adheres to the nomenclature for DiGeorge syndrome as described in GeneReviews (used to refer to patients presenting a similar clinical phenotype without identifiable genetic origin, or one which differs from a 22q11.2 deletion, see). It must be noted however that several names are used interchangeably in literature. Please consult the product page at www.mrcholland.com for more information on disease nomenclature.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. For use on (un)cultured amniocytes, contamination of the sample with maternal DNA may lead to wrong conclusions.
2. 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).
3. Target probes for the 4q35-qter region, 9q34.3 region, 17p13.3 region, and 22q13.33 region are included for research purposes only and not for diagnostic use.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Burnside RD (2015). 22q11.21 Deletion Syndromes: A review of proximal, central, and distal deletions and their associated features. *Cytogenet Genome Res*. 146:89-99.
2. 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.

Implemented changes in the product description
Version B2-10 – 22 September 2025 (03S) - Product description updated to new template. - Intended purpose updated, specifying the assay is manual. Buccal swabs have been removed as specimen type. Testing of at-risk family members has been added as a function of the device. The term 22q11.2 deletion syndrome is specified to encompass DiGeorge syndrome. The 4q, 9q, and 17p regions and DiGeorge type II have been removed. The function of confirming results from other MLPA assays has been removed. - SNV rs372348692 can affect the probe signal. However, the warning for this SNV present in previous product description versions has been replaced by a general warning for small sequence changes, included in section Precautions and Warnings. - Warning for a ligation site >20 nt away from the nearest exon added for probes 05932-L04872 and 08480-L15878. - Warning for target sequences outside of the known coding region added for probes 01223-L05814, 01232-L17068, and 01234-L00781. - Warning for probe located in or near a GC-rich region added for probe 06787-L07383. - Precaution about disease nomenclature added. - Probemix is now IVDR-certified.

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