Instructions for Use

SALSA® MLPA® Probemix P083 CDH1

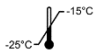
See also the MLPA General Protocol, the product description of the SALSA® MLPA® Reagent Kit and the Coffalyser.Net Reference Manual.

Visit the SALSA® MLPA® Probemix P083 CDH1 product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P083 CDH1**
Version	D2
Catalogue numbers	P083-025R (25 reactions) P083-050R (50 reactions) P083-100R (100 reactions)
Basic UDI-DI	872021148P08362
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 the D1 version, the *CDH1* exon 1 probe has been modified, and two reference probes have been removed.

1. Intended Purpose

The SALSA MLPA Probemix P083 CDH1 is an in vitro diagnostic (IVD)[1] or research use only (RUO) semi-quantitative manual assay[2] for the detection of deletions in the *CDH1* gene in genomic DNA isolated from human peripheral whole blood specimens. P083 CDH1 is intended to confirm a potential cause for, and clinical diagnosis of, diffuse gastric and lobular breast cancer syndrome (DGLBCS), and for molecular genetic testing of at-risk family members.

Copy number variations (CNVs) detected with P083 CDH1 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 *CDH1* gene 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. This device can be used on fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumour tissue for RUO.

[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	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 DGLBCS.	
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 blood-derived DNA samples containing abnormal copy numbers. The standard deviation of each individual probe over all the reference samples was ≤0.10.

A homozygous deletion (copy number 0) of the *CDH1* gene is not expected in blood derived DNA, because such a deletion is associated with embryonic lethality. However, in tumour tissue it can occur.

Please note that the below mentioned final ratios are only valid for germline testing. In a research setting when using DNA isolated from tumour samples, final ratios can be affected both by percentage of tumour cells and by possible subclonality.

Expected Results of Reference Probes

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

Typical Results of Probes Targeting Two Copies (*CDH1*)

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 numbers in normal and affected populations	To determine the expected values in normal and affected populations a study was conducted on over 1,500 MLPA reactions with samples with and without abnormal copy numbers. When the standard deviation of each individual probe over all the reference samples is ≤0.10, the final ratios stated in the table above can be used. Cut-off values were verified with SALSA MLPA Probemix P083 CDH1 in 46 samples from healthy individuals with a normal *CDH1* copy number and three samples with known *CDH1* CNVs. The expected FRs for the corresponding copy number were found in >99% of measurements across 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. 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 P083 CDH1 on one sample with a known *CDH1* CNV and on one sample with a normal *CDH1* copy number. The expected results were obtained using both the lower and upper input amount of DNA.
Interfering substances	SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, Fe^{3+}, EDTA and hemoglobin) can affect the MLPA reaction. A study using SALSA MLPA Probemix P083 CDH1 was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on one sample with a known *CDH1* CNV and one sample with a normal *CDH1* copy number. Tested potential interferents, their potential source, testing concentrations and results are presented in the table below. NaCl and heparin did not interfere with copy number determination. An effect on the FRs was observed for a low number of probes with EDTA and Fe^{3+}, where ambiguous results were obtained for samples harbouring a *CDH1* deletion. However, in the worst case scenario this would lead to delayed results as the assay may need to be repeated. Hemoglobin had the largest effect on the FRs, where both ambiguous and wrong results were observed. Importantly, Coffalyser.Net issued warnings for hemoglobin-affected samples, which would also require the assay to be repeated.

	Interferent / **Source** / **Tested Concentration** / **Results***
	EDTA / Exogenous – specimen collection tubes / 1.5 mM / Expected FR for 88/90 measurements^
	NaCl / Exogenous – DNA extraction / 40 mM / Expected FR for 108/108 measurements
	Fe^{3+} ($FeCl_3$) / Exogenous – DNA extraction / 1 µM / Expected FR for 107/108 measurements
	Heparin / Exogenous – specimen collection tubes / 0.02 U/mL / Expected FR for 108/108 measurements
	Hemoglobin / Endogenous – blood sample / 0.02 µg/ µL / Expected FR for 80/108 measurements
	Blank (TE) / - / - / Expected FR for 108/108 measurements
	* Results are summarized for 18 CDH1 probes across two samples tested in triplicate. ^ Due to technical issues, one of the EDTA triplicate samples with a normal *CDH1* copy number had to be excluded. 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. Experiments on three samples with known *CDH1* CNVs and 46 samples with a normal *CDH1* copy number were carried out using SALSA MLPA Probemix P083 CDH1 to determine whether probes are specific to their target sequence. The expected FRs for the corresponding copy number were found in >99% of measurements across all samples tested.
Accuracy	Results of accuracy are derived from trueness and precision studies. For assay trueness, two previously genotyped samples were tested using SALSA MLPA Probemix P083 CDH1. Two measurements fell outside of the cut-off values for a normal copy number status in probes not affected by the aberration. The FRs were in the ambiguous copy number range, which would, in the worst case scenario, lead to delayed results as the assay may need to be repeated. For all remaining measurements (97%), expected results were obtained. For assay precision, one sample with a known *CDH1* CNV and one sample with a normal *CDH1* copy number were repeatedly tested over multiple days, and by multiple operators. Results showed a correct call in 108/108 (between replicates), 324/324 (between days) and 324/324 (between operators) data points, leading to a precision of 100%.
Clinical validity*	An estimated 10-40% of those who meet the clinical criteria for DGLBCS have pathogenic variants in the gene. Of these, 5-18% are caused by deletions in the *CDH1* gene that can be detected using gene-targeted copy number variation analysis (GeneReviews). * Based on a 2007-2024 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	Target	Exon	Distance to next probe	Length (nt)	Probe number	Warnings
16q13	*ADGRG1*		11.1 **M**b	154	10195-L10655	¬
16q22.1	*CDH1*	Upstream	3.1 kb	138	20903-L28968	Ø
16q22.1	*CDH1*	Exon 1	0.6 kb	242	20908-L32322	
16q22.1	*CDH1*	Intron 1 (Exon 2)	0.4 kb	391	12657-L14803	Ø
16q22.1	*CDH1*	Exon 2	63.4 kb	193	12654-L19369	
16q22.1	*CDH1*	Exon 3	6.7 kb	260	20909-L28974	
16q22.1	*CDH1*	Exon 4	0.2 kb	364	12656-L19936	
16q22.1	*CDH1*	Exon 5	1.5 kb	301	02407-L01853	
16q22.1	*CDH1*	Exon 6	1.5 kb	329	02408-L13240	
16q22.1	*CDH1*	Exon 7	0.4 kb	160	02409-L19374	
16q22.1	*CDH1*	Exon 8	1.2 kb	148	12653-L19725	
16q22.1	*CDH1*	Exon 9	2.3 kb	166	20905-L30071	
16q22.1	*CDH1*	Exon 10	3.7 kb	202	20906-L28971	
16q22.1	*CDH1*	Exon 11	2.9 kb	232	20907-L29534	
16q22.1	*CDH1*	Exon 12	1.3 kb	382	16885-L19718	
16q22.1	*CDH1*	Exon 13	4.7 kb	265	02413-L19371	
16q22.1	*CDH1*	Exon 14	1.5 kb	283	02414-L01860	
16q22.1	*CDH1*	Exon 15	3.7 kb	316	02415-L19372	
16q22.1	*CDH1*	Exon 16	1.6 kb	181	21392-L01862	
16q22.1	*CDH1*	Exon 16	1.1 kb	337	20910-L28975	
16q22.1	*CDH1*	Downstream	13.0 **M**b	172	20904-L29487	Ø
16q23.2	*PLCG2*			216	18052-L29486	¬
2p	Reference - *PPM1B*			274	17873-L22132	
2p	Reference - *DYSF*			348	08802-L26551	
2q	Reference - *SCN2A*			186	06587-L30000	
4p	Reference - *ATP8A1*			130	19616-L26704	
6p	Reference - *PKHD1*			142	10679-L11261	
6q	Reference - *LAMA2*			250	14971-L29482	
9q	Reference - *PCSK5*			292	08722-L28962	
10p	Reference - *NEBL*			225	10244-L15878	
11p	Reference - *PAX6*			357	03092-L30095	
12p	Reference - *KCNA1*			208	11611-L12371	
15q	Reference - *SPG11*			400	09770-L28964	
19p	Reference - *CACNA1A*			310	09065-L09234	
19q	Reference - *PRPF31*			372	06016-L21128	

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 *CDH1* 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.
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

¬ These probes are flanking probes, included to help determine the extent of a deletion. Copy number alterations of flanking probes are unlikely to be related to the condition tested.

Ø These probes target sequences outside of the known coding region.

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. Copy number alterations of reference probes are unlikely to be related to the condition tested.
5. When using DNA samples isolated from tumour tissues (for Research Use Only), reference probes are more prone to have deviating copy number results as compared to blood-derived germline samples. When regions targeted by reference probes are affected by copy number alterations, it can help to turn the slope correction off in Coffalyser.Net analysis to get the correct copy number interpretation on the target region.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations
1. When used on tumour DNA (for Research Use Only): MLPA analysis on tumour samples provides information on the average situation in the cells from which the DNA sample was purified. Gains or losses of genomic regions or genes may not be detected if the percentage of tumour cells is low. In addition, subclonality of the aberration affects the final ratio of the corresponding probe. Furthermore, there is always a possibility that one or more reference probes *do* show a copy number alteration in a patient sample.
2. When used on tumour DNA (for Research Use Only), a lower quality of the extracted DNA can result due to deviations in preanalytical steps (e.g. sample storage conditions). This might result in higher probe standard deviation. Warnings during the Fragment Analysis using Coffalyser.Net will indicate that the MLPA experiment was not optimal on the specific sample(s) used.

Technique-specific limitations
See the MLPA General Protocol.

Implemented changes in the product description

Version D2-05 – 25 September 2025 (03S)
- Product description was updated to new template.
- Intended purpose updated: duplications in *CDH1* removed; 'manual' added to the assay description; syndrome nomenclature changed from hereditary diffuse gastric cancer and/or lobular breast cancer to diffuse gastric and lobular breast cancer syndrome (DGLBCS); use for FFPE tumour tissue added for RUO; added specification that device should not be used for pre-implantation or prenatal testing.
- Exon numbering for probe 12657-L14803 updated.
- Warning for probes targeting a sequence outside of the known coding region included for CDH1 probes 20903-L28968, 12657-L14803 and 20904-L29487.
- Warning for probe 20908-L32322 being more variable and sensitive to certain experimental variations was removed. The probe targeting exon 1 was redesigned in version D2 and the alteration eliminated the previously observed variability.
- Probemix is now IVDR certified.