Instructions for Use
SALSA® MLPA® Probemix P091 CFTR

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 P091 CFTR product page on our website to find Certificates of Analysis and a list of related products.

Product Name	**SALSA® MLPA® Probemix P091 CFTR**
Version	D2
Catalogue numbers	P091-025R (25 reactions) P091-050R (50 reactions) P091-100R (100 reactions)
Basic UDI-DI	872021148P0915Z
Ingredients	Synthetic oligonucleotides, oligonucleotides purified from bacteria, Tris-HCl, EDTA

Regulatory Status	
IVD	EUROPE CE 2797 COLOMBIA ISRAEL
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 D1, one CFTR probe was adjusted. No change in sequence detected.

1. Intended Purpose

The SALSA MLPA Probemix P091 CFTR is an in vitro diagnostic (IVD)[1] or research use only (RUO) semiquantitative manual assay[2] to be used with DNA isolated from human peripheral whole blood specimens. P091 CFTR is intended for the detection of deletions or duplications in the *CFTR* gene and the wild type allele of the *CFTR* p.Phe508del and p.Ile507del mutations in order to confirm a clinical diagnosis and potential cause for cystic fibrosis or congenital absence of the vas deferens (CAVD). Furthermore, the probemix can also be used for carrier screening and for molecular genetic testing of at-risk family members. Carriers of cystic fibrosis or CAVD have a heterozygous *CFTR* variant. Since only CNVs and the above mentioned mutations can be detected with P091 CFTR, using an additional method is recommended to exclude the possibility of an undetected variant.

Copy number variations (CNVs) detected with P091 CFTR 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 *CFTR* gene are point mutations, which will not be detected by MLPA, with the exception of the two aforementioned mutations. 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, 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	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: • 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 cystic fibrosis or congenital absence of the vas deferens (CAVD).	
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.

Expected Results of Reference Probes

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

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

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.

Possible Results of the Wild Type-Specific Probe

Final Ratio (FR)	Mutation Status
0	Homozygous p.Phe508del, p.Ile507del, or (partial) exon 11 deletion, or compound heterozygosity among these mutations.
0.40 - 0.65	A heterozygous mutation of either p.Phe508del or p.Ile507del, or a heterozygous (partial) exon 11 deletion is detected.
0.80 - 1.20	Neither the p.Phe508del/p.Ile507del mutations, nor a partial exon 11 deletion is detected.

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 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 can be used. Cut-off values for copy number determination were verified with SALSA MLPA Probemix P091 CFTR in 70 samples from healthy individuals with normal copy number and six samples with known CNVs. The expected FRs for the corresponding copy number were found in all samples tested with exception of the sample with a heterozygous *CFTR* triplication in which the FR of 1 probe fell slightly below the cut-off range. However, since the result of only one probe was ambiguous the correct genotype can be established in this and all other samples tested.
Expected values for point mutation detection in normal and affected populations	The wildtype-specific probe will only generate a signal when the p.Phe508del/p.Ile507del mutations are absent. This probe detects the wild type allele of the p.Phe508del/P.Ile507del mutations and will give a reduced signal when either of these mutations or a (partial) deletion of exon 11 in the *CFTR* gene is present. This means that a FR of ~0.5 can be caused by a heterozygous mutation of either p.Phe508del or p.Ile507del or a heterozygous (partial) deletion of exon 11. A FR of 0 can correspond to a homozygous mutation of either p.Phe508del or p.Ile507del, a homozygous (partial) deletion of exon 11, or compound heterozygosity for the mutations or for a mutation and a deletion. The expected FR for the wildtype-specific probe was verified with P091 using eight mutation positive samples and 70 samples from healthy individuals and the expected FR was found in all tested samples.
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 P091 CFTR on 4 samples with known CNVs/mutations and on 1 sample without any mutation. The sample with a heterozygous *CFTR* triplication showed minor deviations to the expected FR values with both lower and upper input DNA. This is acceptable, since the heterozygous triplication is detected by 30 probes targeting *CFTR*. Therefore, a few probes with an ambiguous FR would not lead to false results as the results of all probes targeting the *CFTR* gene need to be evaluated together and can be used to confirm each other. All other samples gave the expected results using both the lower and upper input amount of DNA.

<table>
<tr><td>Interfering substances</td><td>SNVs or other polymorphisms (e.g. indels) in the DNA target sequence and impurities in the DNA sample (e.g. NaCl, EDTA and hemoglobin) can affect the MLPA reaction.

A study using SALSA MLPA Probemix P091 CFTR was performed to assess the potential for interference of endogenous and exogenous substances on genomic DNA on samples with known CNVs/mutations. For most probes, 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>Copy number: Expected FR for 400/450 measurements
Mutation: Expected FR for 15/15 measurements</td></tr>
<tr><td>NaCl</td><td>Exogenous – DNA extraction</td><td>40 mM</td><td>Copy number: Expected FR for 423/450 measurements
Mutation: Expected FR for 15/15 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 412/450 measurements
Mutation: Expected FR for 15/15 measurements</td></tr>
<tr><td>Heparin</td><td>Exogenous – specimen collection tubes</td><td>0.02 U/mL</td><td>Copy number: Expected FR for 417/450 measurements
Mutation: Expected MR for 15/15 measurements</td></tr>
<tr><td>Hemoglobin</td><td>Endogenous – blood sample</td><td>0.02 µg/µl</td><td>Copy number: Expected FR for 435/450 measurements
Mutation: Expected MR for 15/15 measurements</td></tr>
</table>
* Results are summarised for all probes across all 5 samples tested.

In two samples deviating FRs for copy number were observed across all conditions including ambiguous results in the blank, however the deviations were more pronounced in the presence of the interfering substances (in particular EDTA, $FeCl_3$ and Hemoglobin). Coffalyser.Net issues warnings for the samples in which the interferent showed an effect, as well as lowered quality scores, which means that the samples need to be re-tested according to the P091 CFTR IFU.

The other three samples did not present with any deviating results even in the presence of interfering substances.

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.</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 to determine whether probes are specific to their target sequence and all probes 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, 13 previously genotyped samples were tested using P091 CFTR and found to have the expected results. Assay precision was tested by repeatedly testing samples with known copy number/mutations over multiple days, and by multiple operators. Results showed a correct call in 2181/2220 data points for copy number and 73/74 data point for mutation status, leading to a precision of >97%.</td></tr>
<tr><td>Clinical validity*</td><td>CFTR: less than 3% of cystic fibrosis is caused by large deletions and duplications in CFTR (Erdogan et al. 2021; Farahzadi and Akbari 2020; Lucarelli et al. 2017; Paracchini et al. 2008; Schneider et al. 2007; Shen et al. 2023; Terzic et al. 2019). However, the frequency of large deletions and duplications can vary between ethnic groups and a number of publications indicate that large deletions and duplications could be more frequent in certain populations.

The p.Phe508del mutation accounts for approximately 66% of all identified mutant CFTR alleles worldwide. The p.Ile507del mutation constitutes ~0.5-1.3% of all the CFTR mutations identified (Bobadilla et al. 2002). The P091 CFTR probemix is not able to distinguish between the p.Phe508del and p.Ile507del mutations, but can indicate the presence of both in a sample.

*(Based on a 2000-2024 literature review)</td></tr>
</table>

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	Mutation	Length (nt)	Probe number	Warnings
7q31.2	*ASZ1*		57.1 kb		136	03571-L03264	¬
7q31.2	*CFTR*	Upstream	0.5 kb		373	12113-L23339	Ø
7q31.2	*CFTR*	Upstream (Exon 1)	0.1 kb		154	02944-L02376	Ø
7q31.2	*CFTR*	Exon 1	24.3 kb		238	03839-L03312	
7q31.2	*CFTR*	Exon 2	4.8 kb		199	02946-L13077	
7q31.2	*CFTR*	Exon 3	21.9 kb		220	02947-L02379	
7q31.2	*CFTR*	Exon 4	3.4 kb		247	02948-L02380	
7q31.2	*CFTR*	Exon 5	1.0 kb		346	03840-L03313	
7q31.2	*CFTR*	Exon 6	1.3 kb		274	13916-L15981	
7q31.2	*CFTR*	Exon 7	3.5 kb		301	13627-L15081	
7q31.2	*CFTR*	Exon 8	1.9 kb		337	02951-L13653	
7q31.2	*CFTR*	Exon 9	6.1 kb		400	18198-L22809	
7q31.2	*CFTR*	Intron 9 (Exon 10)	11.4 kb		391	02953-L30560	Ø
7q31.2	*CFTR*	Exon 11	0.1 kb		465	02954-L23460	
7q31.2	*CFTR*	Exon 11	28.2 kb	p.Phe508del and/or p.Ile507del (wildtype)	330	03322-L14978	∞
7q31.2	*CFTR*	Exon 12	2.6 kb		418	02955-L02387	
7q31.2	*CFTR*	Exon 13	0.2 kb		226	18196-L22807	Δ
7q31.2	*CFTR*	Exon 13	1.9 kb		292	03841-L03314	+
7q31.2	*CFTR*	Exon 14	2.6 kb		142	02956-L13079	
7q31.2	*CFTR*	Exon 15	7.9 kb		160	02957-L02389	±
7q31.2	*CFTR*	Exon 16	0.8 kb		178	02958-L02390	
7q31.2	*CFTR*	Exon 17	3.0 kb		206	02959-L13651	
7q31.2	*CFTR*	Exon 18	3.9 kb		364	18197-L22808	
7q31.2	*CFTR*	Exon 19	1.1 kb		256	02961-L02393	
7q31.2	*CFTR*	Exon 20	3.0 kb		283	02962-L02394	
7q31.2	*CFTR*	Exon 21	12.9 kb		310	03576-L13076	
7q31.2	*CFTR*	Exon 22	15.0 kb		355	13629-L15083	±
7q31.2	*CFTR*	Exon 23	10.4 kb		382	02965-L02397	
7q31.2	*CFTR*	Exon 24	11.8 kb		409	02966-L02398	
7q31.2	*CFTR*	Exon 25	0.8 kb		436	02967-L02399	
7q31.2	*CFTR*	Exon 26	1.4 kb		148	03842-L03315	
7q31.2	*CFTR*	Exon 27	0.1 kb		190	03574-L02400	
7q31.2	*CFTR*	Exon 27	58.1 kb		173	03578-L02939	
7q31.2	*CTTNBP2*				445	03572-L03267	¬
1p	Reference				184	14817-L16525	
5q	Reference				130	00797-L00463	
11p	Reference				474	02757-L02206	
12p	Reference				319	17454-L21210	
13q	Reference				167	16255-L19128	
15q	Reference				454	00605-L00018	
17q	Reference				427	13981-L15550	
19p	Reference				265	02318-L01809	
20q	Reference				481	16290-L18582	
21q	Reference				214	03791-L05919	

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 *CFTR* 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. Annotations of several probes with targets at the edge of or slightly outside the coding region, were 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. A lowered probe signal can be due to the p.Phe508del mutation, the p.Ile507del mutation, or a partial deletion of exon 11. The p.Phe508Cys mutation (F508C; c.1523T>G; rs74571530) is not expected to influence the signal of this probe. Other variants near the ligation site can also cause a lowered signal. A positive result must be confirmed by another method.

¬ 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.

Δ This probe may be sensitive to certain experimental variations. Aberrant results should be treated with caution.

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

\+ 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.

± The p.Trp846X mutation (W846X; rs397508393 or rs267606722) could influence the signal of the 160 nt probe. The c.3528delC mutation (3659delC;

rs78984783) could influence the signal of the 355 nt probe. In case of apparent deletions, it is recommended to sequence the region targeted by these probes

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. Deletion of duplication of the flanking genes *ASZ1* and *CTTNPB2* are not expected to be the cause of cystic fibrosis or CAVD. These probes have only been included to delineate the extent of large deletions and duplications.
6. The probe at 330 nt detects the wild type allele of the p.Phe508del and p.Ile507del mutations. A final ratio of ~0.5 can be caused by a heterozygous mutation of either p.Phe508del or p.Ile507del or a heterozygous (partial) deletion of exon 11. A final ratio of 0 can correspond to a homozygous mutation of either p.Phe508del or p.Ile507del, a homozygous (partial) deletion of exon 11, or compound heterozygosity for the mutations or for a mutation and a deletion.

Technique-specific precautions
See the MLPA General Protocol.

8. Limitations

Probemix-specific limitations

1. SALSA MLPA Probemix P091 CFTR cannot discriminate between p.Phe508del, p.Ile507del and a partial deletion of *CFTR* exon 11, as all three mutations will lead to an equal reduction in signal of the 330 nt probe.

Technique-specific limitations
See the MLPA General Protocol.

9. References Cited in this IFU

1. Bobadilla JL et al. (2002). Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. *Hum Mutat*. 19:575-606.
2. Erdogan M et al. (2021). The Genetic Analysis of Cystic Fibrosis Patients With Seven Novel Mutations in the CFTR Gene in the Central Anatolian Region of Turkey. *Balkan Med J*. 38:357-364.
3. Farahzadi HN and Akbari MT. (2020). Analysis Variants of the CFTR Gene in Iranian Cystic Fibrosis Patients. *J Human Gen Genom.* 4:e123474.
4. Lucarelli M et al. (2017). A new targeted CFTR mutation panel based on next-generation sequencing technology. *J Mol Diagn*. 19:788-800.
5. Paracchini V et al. (2008). Molecular and clinical features associated with CFTR gene rearrangements in Italian population: identification of a new duplication and recurrent deletions. *Clin Genet*. 73:346-352.
6. Schneider M et al. (2007). Large deletions in the CFTR gene: clinics and genetics in Swiss patients with CF. *Clin Genet*. 72:30-38.
7. Shen Y et al. (2023). Genetic spectrum of Chinese children with cystic fibrosis: comprehensive data analysis from the main referral centre in China. *Journal of Medical Genetics*. 60:310-315.
8. Terzic M et al. (2019). Cystic fibrosis mutation spectrum in North Macedonia: A step toward personalized therapy. *Balkan J Med Genet*. 22:35-40.

Implemented changes in the product description

Version D2-06 – 19 June 2025 (03S)

- Intended purpose updated to specify that P091 is a manual assay and that it can be used for carrier screening.
- Description of probe targets at the edge of or slightly outside the coding region has been adjusted. No change in actual target sites.
- Warnings for a ligations site >20nt from the nearest exon were added for probe 03841-L03314.
- SNVs rs397508393, rs267606722 and rs78984783 can affect the probe signal. However, the warnings for these SNVs present in previous product description versions have been replaced by a general warning for small sequence changes, included in section Precautions and Warnings.
- Warnings for probes targeting sequences outside of the known coding region were added for 12113-L23339, 02944-L02376 and 02953-L30560.
- Probemix is now IVDR certified.