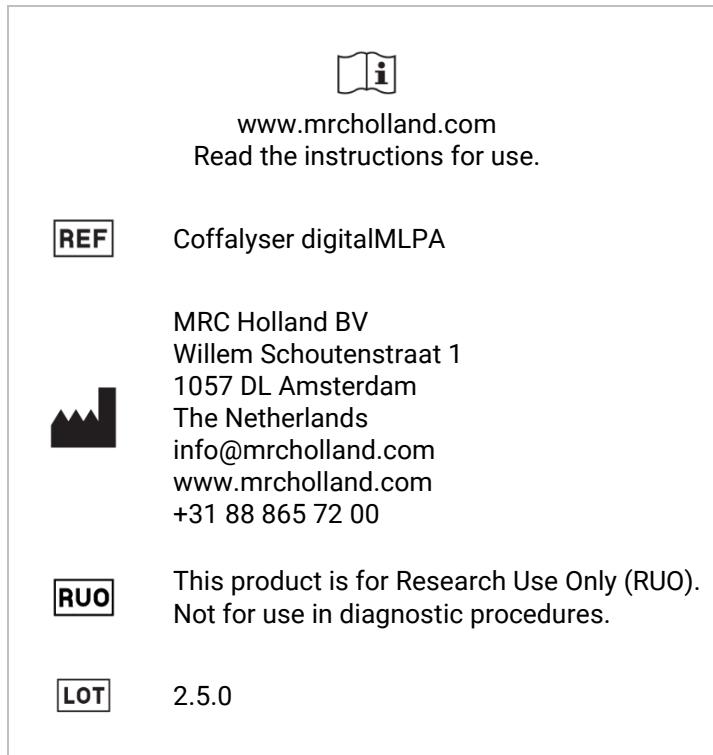




Coffalyser digitalMLPA™ Release Notes



General information

Coffalyser digitalMLPA™ is Research Use Only (RUO) software designed for the analysis of digitalMLPA™ data generated using a NXtec™ probemix* as described in the (MS-)digitalMLPA NXtec Protocol.

* To be used in combination with a NXtec Reagent Kit and one or multiple NXtec barcode plates.

Version numbers

Software version 2.5.0

Module versions:

Configuration	2025-12-01 14:55
Data conversion	2025-06-13 13:36
Fragment analysis	2025-06-13 13:36
Comparative analysis	2025-06-13 13:36
Result analysis	2025-06-13 13:36
Reporting	2025-06-13 13:36

Changes in Coffalyser digitalMLPA compared to previous versions

Table 1 Overview of unexpired releases

			Summary of changes
Releases	v2.5.0	Dec 2025	Major update to all software modules and configuration items for all products. digitalMLPA lots no longer supported in this software release: D001-B1-1119, D001-B1-0421, D001-C1-1221, D007-A1-0922.
	v251008.1227	Oct 2025	Product sheets for lots D002-A1-0225 and D024-A1-0125 added. Product sheets for lots D001-D1-0124, D006-A1-1022 and D007-B1-0124 updated. Minor changes in the configuration module related to barcode collections, lot detection and read assignment to improve performance.
	v241105.1259	Nov 2024	Product sheet for lot D006-A1-1022 updated in configuration module, product sheet for lot D007-B1-0124 added to configuration module
	v240719.0943	July 2024	Product sheet for lot D001-D1-0124 added to configuration module
	v221020.1234	Oct 2022	Product sheets for lots D006-A1-1022 and D007-A1-0922 added to configuration module

Table 2 Overview of changes per probemix lot for unexpired releases

			Releases				
			v221020.1234	v240719.0943	v241105.1259	v251008.1227	v2.5.0
			Oct 2022	July 2024	Nov 2024	Oct 2025	Dec 2025
Product lots	D001	B1-1119	No changes	No changes	No changes	No changes	Not supported ^a
		B1-0421	No changes	No changes	No changes	No changes	Not supported ^a
		C1-1221	No changes	No changes	No changes	No changes	Not supported ^a
		D1-0124	-	New	No changes	Minor update of product sheet.	Major update *
	D002	A1-0225	-	-	-	New	Major update *
		D006	A1-1022	New	No changes	Updated borders in product sheet.	Major update *
	D007	A1-0922	New	No changes	No changes	No changes	Not supported ^a
		B1-0124	-	-	New	Updated borders in product sheet.	Major update *
	D024	A1-0125	-	-	-	New	Major update *

^a See Table 1 for details.

^a Please contact MRC Holland at info@mcholland.com for support.

We recommend to always use the latest version of Coffalyser digitalMLPA that supports your lot. For software and product sheet updates in bold, (re)validation is recommended.

For an overview of changes made in expired software releases, please consult the Release Notes that are included in the Documentation folder of the corresponding software package.

Software version 2.5.0

Configuration: 2025-12-01 14:55 | Data conversion: 2025-06-13 13:36 | Fragment analysis: 2025-06-13 13:36 | Comparative analysis: 2025-06-13 13:36 | Result analysis: 2025-06-13 13:36 | Reporting: 2025-06-13 13:36.

Probemix-specific changes

D001

D1

- Configuration files for lot D1-0124 modified with major software update.
- Adjusted borders for probes with an expected normal copy number of 4. See Product Description for details.
- Small adjustment of the lowest border for probes with an expected normal copy number of 2, to align with the cut-off values described in the Product Description.

C1 & B1 lots are no longer supported*.

D002

A1

- Configuration files for lot A1-0225 modified with major software update.
- Adjusted borders for probes with an expected normal copy number of 4. See Product Description for details.
- Small adjustment of the lowest border for probes with an expected normal copy number of 2, to align with the cut-off values described in the Product Description.

D006

A1

- Configuration files for lot A1-1022 modified with major software update.
- Adjusted borders for probes targeting the X-chromosome in female samples, to be in line with autosomal probe borders. See Product Description for details.

D007

B1

- Configuration files for lot B1-0124 modified with major software update.
- Adjusted borders for probes targeting the X-chromosome in female samples, to be in line with autosomal probe borders. See Product Description for details.

A1 lots are no longer supported*.

D024

A1

- Configuration files for lot A1-0125 modified with major software update.
- Adjusted borders for probes targeting the X-chromosome in female samples, to be in line with autosomal probe borders. See Product Description for details.
- Small adjustment of the lowest border for probes with an expected normal copy number of 2, to align with the cut-off values described in the Product Description.

General software changes

Data analysis:

- Possibility is added to exclude a subset of targets from the analysis (masking).
- Probes targeting the X-chromosome can now be analysed using a mix of male and female reference samples, reducing the total number of reference samples needed.
- Probemix lot detection method was updated.
- Functionality for the analysis of methylation-specific digitalMLPA data was added.

* We recommend to always use the latest version of Coffalyser digitalMLPA that supports your lot. Please contact MRC Holland at info@mrcholland.com for support.

- Sample populations used for inter-normalisation and comparison to the expected range are now defined separately, to enable tailored settings for these populations per probe.
- Results are now rounded before comparing them to borders for aberrant calling.
- Analysis without dedicated reference samples is now blocked for probemixes for which this type of analysis is not appropriate.
- Coffalyser Definition Files (*.cdf) generated with the previous software version are compatible with this software version 2.5.0.
- Coffa files generated with previous versions of the software are *not* compatible with this software version 2.5.0.

Quality assessment:

- Plausible root cause is provided in case of quality warnings for a sample.
- Warning is displayed in the Quality Report in case of low DNA input. Estimated DNA quantity is not available anymore.
- Reference Sample Quality (RSQ) check was updated and is now available on probe level. In addition, separate warnings are now issued for variability in the populations for inter-normalisation and comparison, respectively nRSQ and cRSQ.
- A check on probe signal (intra ratio) in the reference samples was added: nRSQ (MIRR) and cRSQ (MIRR).
- The check on the number of available reference samples was updated and is now available on probe level: nRSQ (qty) and cRSQ (qty).
- Sample can now fail if a user-defined reference sample was excluded from the analysis due to quality issues: nRSQ (excl).
- Warning on probe level is given in case the probe result falls in the ambiguous range according to the set boundaries for the probe: Ambiguous.
- Warning on probe level is given in case the probe result is aberrant and the confidence intervals of the probe result and the reference sample population for comparison are overlapping: Inconclusive.
- Reference Probe Quality (RPQ) check was updated, making it less sensitive to single reference probe deviations.
- Quality checks on no-DNA samples were introduced, to enable quick interpretation. Failed no-DNA samples now lead to a warning for DNA samples in the same experiment.
- Sample can now fail if detected sex does not match the sample sex defined by the user.
- A check on barcode plate lot compatibility with the probemix lot was added.
- A check on identical read length was added, to prevent skewed results introduced by adapter trimming during FASTQ file generation.

Reporting:

- Quality Report (PDF report) is now generated for each experiment, containing general quality information for all samples, and detailed quality information, including a plausible root cause, for samples with detected issues.
- Results (PDF report), previously called Sample Results 1.pdf, was updated:
 - o Major textual and lay-out changes.
 - o Results are now grouped into (sub)panels.
 - o Charts are available per panel for specific applications.
 - o Statistical information on the reference sample population used for comparison is available for each probe.
 - o A table with detailed information on Reference Sample Quality is displayed in case at least one RSQ warning was issued for the sample.
 - o Probe result is now called aberrant 'yes' or 'no' based on the expected range and significance is shown in case the result is outside the expected range.
- Excel Report (Excel file) was extensively updated and now also contains quality information and probe warnings.
- No-DNA (PDF report) is generated for no-DNA sample and includes a summary of the quality checks performed.
- XML output can be generated per sample.

- Overview.pdf and General Details.pdf are no longer available, as information from these reports is now present in the above-mentioned output files.

Configuration:

- Improved robustness of probemix lot detection.
- Read assignment to probes is updated to be more consistent between probemixes.

Other:

- About Page available in the software.
- Software version numbering system updated to Major.Minor.Patch.

Related to the Coffalyser digitalMLPA Release Notes:

- Expired software releases (before October 2022) removed.

Software version 251008.1227

Configuration: 2025-10-08 12:27 | Data conversion: 2021-10-19 09:58 | Fragment analysis: 2021-10-19 09:58 | Comparative analysis: 2021-10-19 09:58 | Extended analysis: n/a | Reporting: 2021-10-19 09:58.

General

Configuration:

- Configuration modified to accommodate new commercially available NXtec probemixes.
- Barcode collections updated to be compatible with XLEAP-SBS chemistry.
- Previous limited-release barcode collection removed from the configuration module.
- Improved lot detection in DNA samples, in particular for large probemixes and oncogenetic applications.
- Improved lot detection in no-DNA samples in combination with XLEAP-SBS chemistry.
- Improved read assignment in combination with XLEAP-SBS chemistry.

Related to the Coffalyser digitalMLPA Release Notes:

- Product names were updated in the General Information from SALSA® digitalMLPA™ to NXtec™.

Probemix-specific

D001-D1:

- Minor update of product sheet for lot D001-D1-0124, including a small adjustment in DNA contamination detection and SNP code analysis for sample identification.

D001-C1:

- Product sheet for lot D001-C1-1221 remains unchanged.

D001-B1:

- Product sheets for lot D001-B1-1119 and D001-B1-0421 remain unchanged.

D002-A1:

- Product sheet released for lot D002-A1-0225 for research use only (RUO).

D006-A1:

- Product sheet for lot D006-A1-1022 was updated with adjusted borders as well as a minor adjustment in SNP code analysis for sample identification. See Product Description for details.

D007-A1:

- Product sheet for lot D007-A1-0922 remains unchanged.

D007-B1:

- Product sheets for lot D007-B1-0124 were updated with adjusted borders as well as a minor adjustment in SNP code analysis for sample identification. See Product Description for details.

D024-A1:

- Product sheet released for lot D024-A1-0125 for research use only (RUO).

Software version 241105.1259

Configuration: 2024-11-05 12:59 | Data conversion: 2021-10-19 09:58 | Fragment analysis: 2021-10-19 09:58 | Comparative analysis: 2021-10-19 09:58 | Extended analysis: n/a | Reporting: 2021-10-19 09:58.

General

- Configuration module version modified to accommodate new commercially available digitalMLPA probemixes.

Probemix-specific

D001-D1:

- Product sheet for lot D001-D1-0124 remains unchanged.

D001-C1:

- Product sheet for lot D001-C1-1221 remains unchanged.

D001-B1:

- Product sheets for lot D001-B1-1119 and D001-B1-0421 remain unchanged.

D006-A1:

- Product sheet for lot D006-A1-1022 was updated with adjusted borders, see Product Description for details.

D007-A1:

- Product sheet for lot D007-A1-0922 remains unchanged.

D007-B1:

- Product sheets released for lot D007-B1-0124 for research use only (RUO): *D007-B1 default product sheet* and *D007-B1 near haploid_low hypodiploid product sheet*.

Software version 240719.0943

Configuration: 2024-07-19 09:43 | Data conversion: 2021-10-19 09:58 | Fragment analysis: 2021-10-19 09:58 | Comparative analysis: 2021-10-19 09:58 | Extended analysis: n/a | Reporting: 2021-10-19 09:58.

General

- Configuration module version modified to accommodate new commercially available digitalMLPA probemixes.

Probemix-specific

D001-D1:

- Product sheet released for lot D001-D1-0124 for research use only (RUO).

D001-C1:

- Product sheet for lot D001-C1-1221 remains unchanged.

D001-B1:

- Product sheets for lot D001-B1-1119 and D001-B1-0421 remain unchanged.

D006-A1:

- Product sheet for lot D006-A1-1022 remains unchanged.

D007-A1:

- Product sheets for lot D007-A1-0922 remain unchanged.

Software version 221020.1234

Configuration: 2022-10-20 12:34 | Data conversion: 2021-10-19 09:58 | Fragment analysis: 2021-10-19 09:58 | Comparative analysis: 2021-10-19 09:58 | Extended analysis: n/a | Reporting: 2021-10-19 09:58.

General

- Configuration module version modified to accommodate new commercially available digitalMLPA probemixes.
- Product sheets for previous limited-release probemix versions removed from the configuration module.

Probemix-specific

D006-A1:

- Product sheet released for lot D006-A1-1022 for research use only (RUO).

D007-A1:

Product sheets released for lot D007-A1-0922 for research use only (RUO): *D007-A1 default product sheet* and *D007-A1 near haploid_low hypodiploid product sheet*.

D001-C1:

- Product sheet for lot D001-C1-1221 remains unchanged.

D001-B1:

- Product sheets for lot D001-B1-1119 and D001-B1-0421 remain unchanged.