Mentype® AMLplus Profiler

Handbook

This software is an integral component of the RUO Mentype® AMLplus Mutation Analysis Kit and is not intended for standalone use.

APMSWHB01v1en
17.07.2026

REF BT00005SW

LOT Version v1.0.1 and higher

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Notice of Change

Please note the following adaptions compared to the previous handbook version:

Document code	Changes	Date
APMSWHB01v1en	Initial version	17.07.2026

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Content

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Product description

The Mentype® AMLplus Profiler software is an integral, non-standalone component of the **Mentype® AMLplus Mutation Analysis Kit** and is intended for its data analysis and evaluation. The software evaluates genomic targets, associated with Acute Myeloid Leukemia (AML), in a qualitative and/or semi-quantitative manner. The data input for the software originates from data analyzed with Thermo Fisher Scientific GeneMapper™ or GeneMapper™ ID-X software.

Scientific background

Acute Myeloid Leukemia (AML) is a biologically heterogeneous disorder of the hematopoietic system characterised by the accumulation of immature myeloid cells. At the molecular level, AML-associated samples exhibit a wide spectrum of recurrent genetic alterations affecting pathways involved in cell proliferation, differentiation, and metabolism [1]. The identification and analysis of such molecular alterations have therefore become central to research efforts aimed at understanding disease mechanisms and supporting the development and evaluation of targeted therapeutic approaches.

Materials provided

The Mentype® AMLplus Profiler is available via download at:

www.biotype.de/en/software

The **password** required for the download is included in the kit upon delivery.

NOTE

Security patches and software updates must be applied as soon as they become available. End-users will be informed of all updates of the Mentype® AMLplus Profiler, which are mandatory to implement.

Material and devices required but not provided

All materials, devices, reagents, and kits necessary for the correct application of the Mentype® AMLplus Mutation Analysis Kit shall be used. Refer to the assay handbook for detailed instructions. The Mentype® AMLplus Profiler, as an integral component of the kit, shall only be used for analysis of data generated with this assay.

System requirements

Table 1. System requirements for the Mentype® AMLplus Profiler

Specification	System requirement
Operating system	Windows 11
Processor	2 GHz Dual-Core
Free hard disk	500 MB
Minimum RAM	2 GB

Antivirus Software: Up-to-date antivirus software must be installed and active on the host computer.

Network: A network connection is not required for operation. Where the host computer is connected to a network, the laboratory's standard network and access protections apply.

Warnings and precautions

- Read the handbook carefully before using the product.
- This software does not replace the visual inspection and plausibility assessment of raw data in GeneMapper™. It serves only as a supplementary tool to automate and simplify target evaluation.
- Before the first use, check the system requirements. Consult your local IT for installation procedures and refer to chapter Installation. Administrator rights are needed for installation.

- The user is responsible for installing the application in a secure environment with regard to the operating system, network and data backup and for taking appropriate cybersecurity measures.
- If personalized access to the software is unauthorized or restricted, please contact the software administrator.
- The software does not archive sample analyses. File storage and backup of raw data and reports generated are the responsibility of the laboratory professional.

Installation

NOTE

Administrator rights are required to install the Mentype® AMLplus Profiler software.

Running the installer

1	Launch the installer
	▪ Locate the downloaded .exe file. ▪ Double-click the file to start the installation
2	Automatic detection of previous versions
	Upon launch, the installer will: ▪ Automatically scan the system for older installed versions. ▪ If an older version is found, you will be notified and asked for agreement to uninstall older versions. The installer will automatically remove the old version before continuing. No further manual action is required.
3	Welcome Screen
	▪ Click **Next** to proceed.
4	Select installation location
	▪ Choose the destination folder. Default location (recommended): C:\Programs\Biotype\AMLplus_Profiler_RUO ▪ Click **Next**.

5	Select additional tasks
	▪ You may be prompted to: Overwrite the installation folder if it already exists or create a Desktop shortcut ▪ Select the desired options and click **Next**.
6	Ready to install
	▪ Review your installation settings. ▪ Click **Install** to begin the installation.
7	Completion
	▪ Once installation is complete, click **Finish**. ▪ Optionally, check Launch Mentype® AMLplus Profiler to start the application immediately.

Finalizing the installation, the software can be launched via desktop shortcut or start menu.

Software updates

When installing a newer version of the software, simply run the new installer. The installer will automatically detect the existing version, uninstall it, and install the updated version. No manual uninstallation is required.

User Interface

The Mentype® AMLplus Profiler software is used for the analysis and evaluation of GeneMapper™ TXT output files generated using the Mentype® AMLplus Mutation Analysis Kit.

The user interface supports:

- Import of experimental run data (TXT)
- Automated evaluation of control samples
- Assessment of internal and amplification controls
- Variant detection analysis
- Structured display of results for professional interpretation
- Report generation as TXT or pdf

Start screen and data import

Upon launching the software application, the **Start Screen** is displayed automatically. From this screen, the user may import a GeneMapper™ output file (TXT format) containing experimental run data. For a detailed workflow refer to chapter Short protocol data analysis.

Following successful import, the software performs an automated analysis and displays the **Result View**. Only validated GeneMapper™ TXT files generated in accordance with the Mentype® AMLplus Mutation Analysis Kit handbook shall be imported.

Result View

The Result View consists of three functional sections: Run Validity Table, Run Results Table (see Figure 1) and Details View (see Figure 2). The Sample ID always displays the *Sample File Name* as defined within the Data collection software of the Genetic Analyzer used.

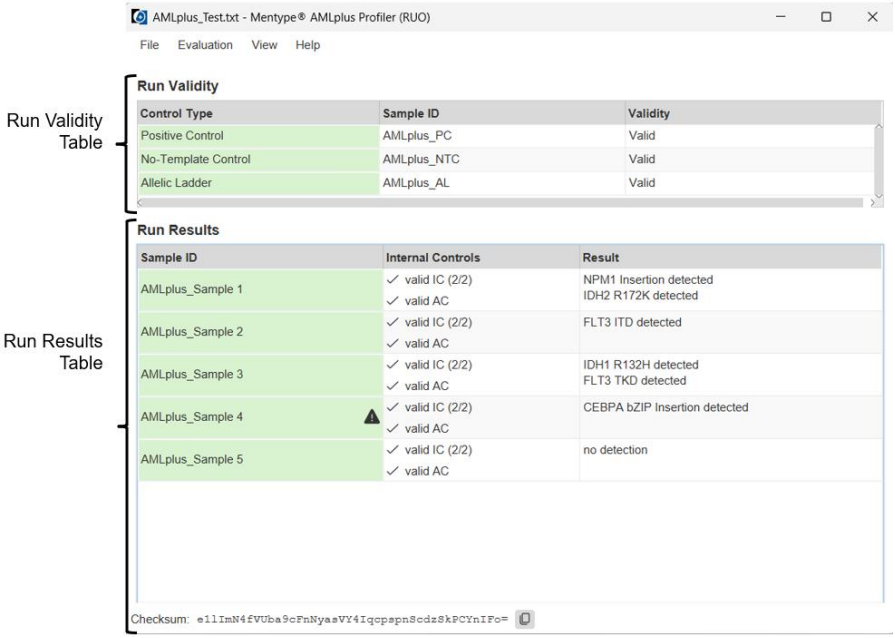


Figure 1. Result View

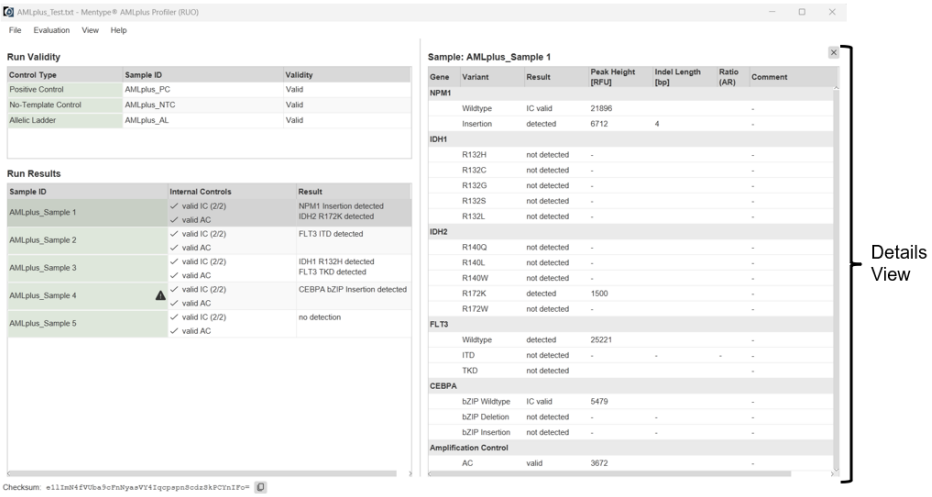


Figure 2. Details View

The table of run validity and run results presents evaluation outcomes in textual format, with additional color coding provided as a visual aid (see Table 2).

Table 2. Display of evaluation results

valid	Green background for valid controls and samples
invalid	Red background for invalid controls and samples
⚠	Green background with warning triangle for valid controls or samples with additional comments or warning messages to be considered

NOTE

Validity assessment is performed in accordance with performance characteristics of the Mentype® AMLplus Mutation Analysis Kit. Please refer to the kit handbook for further details.

Run Validity Table

The **Run Validity Table** displays the evaluation results of mandatory control samples contained in the imported experimental data for the Positive Control PC (*Mentype® AMLplus Positive Control*), the No-Template Control NTC and the Allelic Ladder AL (*Mentype® AMLplus Allelic Ladder*). To display detailed information for a specific control, click the corresponding row. The information will appear in the **Details View**.

NOTE

If any control is marked as invalid, no further assessment of the samples is performed. In this case, all samples are considered invalid.

Run Results Table

This table presents the evaluation results for all samples of interest contained in the imported data file. Results of internal controls are seperately listed depending on control type (see Table 3).

Table 3. internal control evaluation

✓ valid IC (2/2) ✕ invalid IC (0/2)	**Internal Control IC**: Template dependent control. Defined by targets ▪ NPM1 Wildtype ▪ CEBPA bZIP Wildtype
✓ valid AC ✕ invalid AC	**Amplification Control AC**: Template independent control. Checks for PCR inhibitors and general PCR performance.

The column “Result” displays the variant detection outcome for each sample at a glance. To review detailed findings for a specific sample, select the corresponding row. The detailed information will be shown in the **Details View**.

Details View

The **Details View** is located on the right-hand side of the user interface. It can be accessed by:

- Selecting a row in the **Run Validity Table** or **Run Results Table**, or
- Navigate to **View → Show/Hide Details** in the menu bar.

All variants included in the Mentype® AMLplus Mutation Analysis Kit are listed and grouped by gene name. The column *Results* shows validity status for Internal Controls (IC) and variants detected/not detected or inconclusive for other targets.

If a variant is detected

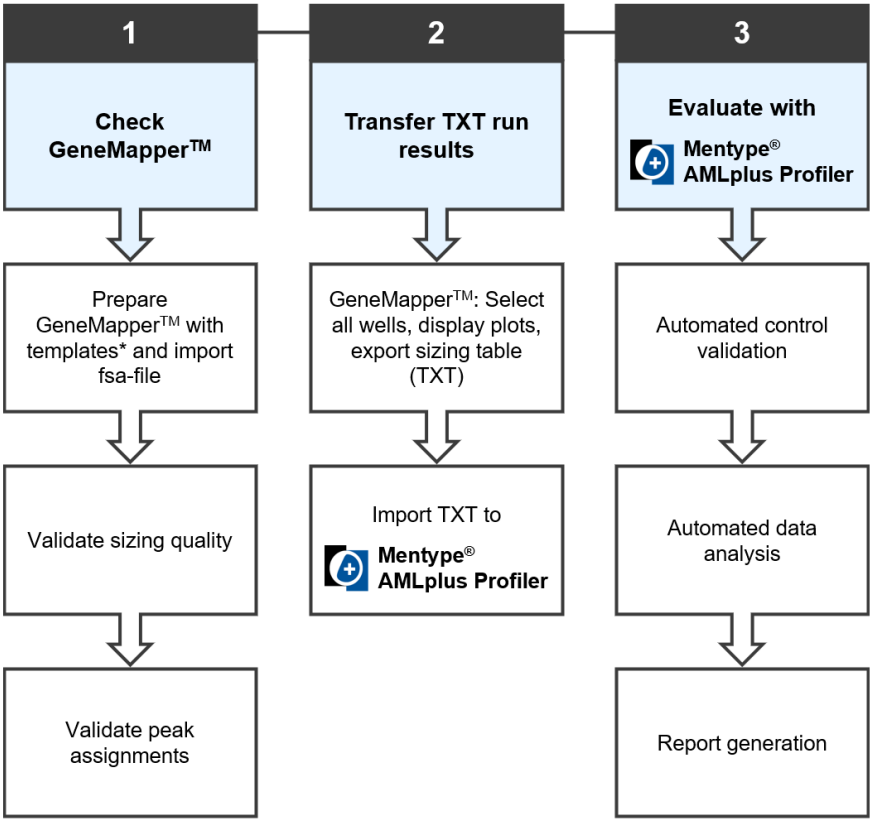
- Peak Height is reported in RFU.
- For insertions or deletions (INDELs), the INDEL Size is reported in base pairs [bp].
- In the case of FLT3 ITD detection, the *Allelic Ratio (AR)* or the *Proportion of Mutant Alleles (PMA)* is calculated and displayed (see chapter Ratio Calculation)

Any warnings or messages are displayed in the column “Comment.”

Procedure

The following procedure outlines the complete data analysis workflow for the Mentype® AMLplus Mutation Analysis Kit, in which the Mentype® AMLplus Profiler represents one component of the overall process.

Workflow overview



Short protocol data analysis

After completion of the capillary gel electrophoresis and data collection of the Mentype® AMLplus Mutation Analysis Kit, proceed with the following data analysis procedure.

1 GeneMapper™ pre-analysis

1.1 GeneMapper™ preparation

Panel Manager
Import the provided template files for Panel, Bins, Stutter (ID-X only)

GeneMapper™ (ID-X) Manager
Import the provided template for Analysis Method, Size Standard and Plot Setting

1.2 FSA-file import

Add Samples to Project
- browse for run folder, select and *Add to List* → *Add*

1.3 Sample analysis

Select the following properties in the appropriate columns of the sample sheet and choose *Analyze*.

Column Name	Select
Sample Type	Allelic Ladder, Positive Control, Negative Control or Sample
Analysis Method	
Panel	Select the previously imported BIOTYPE template
Size Standard	

1.4 Validate sizing quality

- No quality flag for Sizing Quality (SQ)
- Sizing quality of ≥ 0.995 within Size Match Editor
- Allelic ladder peaks harmonized with bin ranges (especially for GeneMapper™ 6)

1.5 Validate peak assignments

- Open Electropherogram (EPG) of the samples
- validate correct peak assignments
- in case of Off-ladder (*OL* or *?*) detections, validate type of peak (artefact vs. misassigned amplicon)

2 Transfer of TXT results

2.1 GeneMapper™ export of sizing table

- Select all wells and samples of the *Samples Table*
- Open the Electropherogram (EPG)
- Choose *File → Export Table* to export the sizing table
- save TXT in desired folder

3 Data evaluation with Mentype® AMLplus Profiler

3.1 Starting Mentype® AMLplus Profiler

- Open the application
- Open the GeneMapper™ TXT file
- Approve or adapt suggested controls for the run (PC, NTC, AL)

3.2 Data Evaluation

- Run and sample validity listed and color coded:
  red – invalid, green – valid,
  warning triagle – closely review comment section of detail view
- Results of each sample are summarized within Run overview or sample-specific in detail view with furthe information

3.3 Report generation

To export results of all or selected samples choose *File → Export to TXT* or *File → Print* (pdf)

NOTE

The GeneMapper™ software must be configured using the appropriate template files, including Analysis Method, Bins, Panels, Stutter (ID-X only) and Plot Settings. Please refer to the handbook for the Mentype® AMLplus Mutation Analysis Kit or download the template files directly from www.biotype.de/en/template-files

NOTE

Peak assignment validation in GeneMapper™ is critical. Double-assignments (except in CEBPA bZIP INDEL or FLT3 ITD) will invalidate the target region. Unassigned peaks are not evaluated by the Mentype® AMLplus Profiler and may lead to invalidation.

Functions

Reporting

PDF report

The Mentype® AMLplus Profiler enables the generation of a structured analysis report in PDF. The report provides documented evidence of the provided run information, the evaluation result of all controls and sample results.

All samples or preselected samples from the **Run Results Table** can be printed. Navigate to **File** → **Print** to generate a report.

A report configuration window opens, allowing the user to define report content and enter additional run information (see Figure 3).

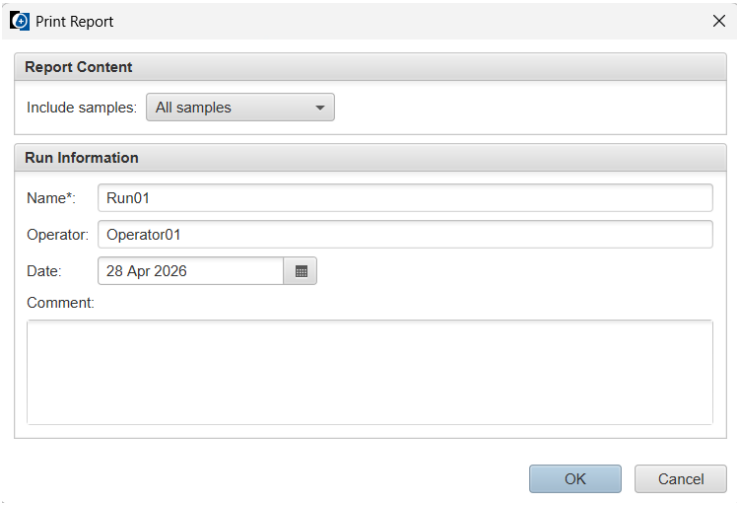


Figure 3. Print report

The report content can be defined using the dropdown menu in the report configuration window.

Table 4. Available options for pdf report content

Option	Report includes
No samples	▪ Run information ▪ Run validity results ▪ Sample overview
All samples	▪ Run information ▪ Run validity results ▪ Complete details for all samples
Selected Samples Only*	▪ Run information ▪ Run validity results ▪ Detailed result sections only for user-selected samples

* Sample selection must be performed in the Run Results Table prior to opening the Print dialog by holding e. g. Ctrl to select individual rows.

The following run information fields are available in the report configuration window:

Table 5. Run information entry for pdf report

Parameter	Comment
Name	mandatory field e. g. run or experiment name
Operator	optional
Date	optional e. g. run or experiment date as desired
Comment	optional e. g. any general comments on run evaluation or similar max. 500 characters

After completing the required fields, select **OK** to proceed.

NOTE

The generated PDF report reflects the analysis state at the time of export. Users shall ensure the correctness of selected samples, completeness of entered run information and proper storage according to laboratory quality management procedures.

NOTE

The software does not automatically archive generated reports. Long-term storage and backup are the responsibility of the laboratory operator.

TXT export

The Mentype® AMLplus Profiler is able to export analysis results in structured TXT format. The TXT export:

- Contains all samples included in the current analysis session
- Includes all alleles/targets defined in the Mentype® AMLplus Mutation Analysis Kit. Both detected and non-detected targets are listed as well as validity status of run and all samples.

- Contains all numerical values as displayed in the Details View (Peak height, INDEL Length where relevant, calculated ratios for FLT3 ITD)
- Contains an event index per detection to uniquely identify all events measured. It is a string composed of [Gene]_E[number] and begins with 1 for each gene.
- Is suitable for import into spreadsheet software (e. g., Microsoft Excel) or Laboratory Information Management Systems (LIMS)

The export function supports downstream data processing, archiving, and integration into laboratory workflows.

Navigate to **File → Export to TXT…** to generate a TXT export. A configuration window opens, allowing the user to define additional run information like experiment or run *Name* (mandatory), *Operator* (optional) and *Date* (optional) information.

Select a desired storage location, enter a file name and click **Save**.

NOTE

The software does not automatically archive exported files. File storage and backup are the responsibility of the laboratory professional.

Ratio Calculation

Navigate to **File → Preferences,** tab **Calculation** to configure the method for FLT3 ratio calculation. Click **OK** to confirm. The selected calculation method is displayed in the **Result Details View** and is included in the generated PDF report.

The following methods can be selected:

- Allelic Ratio (AR): $AR = \frac{AUC_{FLT3\ ITD}}{AUC_{FLT3\ WT}}$
- Proportion of mutant Alleles (PMA): $PMA = \frac{AUC_{FLT3\ ITD}}{AUC_{FLT3\ WT} + AUC_{FLT3\ ITD}}$

AUC… Area under the curve

If several clones of FLT3 ITD are detected, both methods use the sum of AUC for all insertions detected.

System default setting is Allelic Ratio (AR).

NOTE

After changing the calculation method, any previously uploaded TXT file must be re-evaluated using **Evaluation → Evaluate…**

Date and number format

Navigate to **File → Preferences,** tab **General** to select the desired regional format for date and numeric representation to be applied in the user interface, PDF report and TXT data export. Confirm with **OK**.

Table 6. Available date and time formats

Format	Example date	Example time	Example number
English (United Kingdom)	17 Jul 2026	14:30	1234.56
English (United States)	Jul 17, 2026	2:30 PM	1234.56
German	17.07.2026	14:30	1234,56
ISO 8601	2026-07-17	14:30	1234.56

System default setting is the international standard ISO 8601.

NOTE

Note that LIMS or other downstream software systems rely on e. g. fixed date formats, specific decimal separators and region-dependent number formatting

Exported data may not be displayed accordingly if the format setting is modified.

Troubleshooting

The software automatically generates log files documenting relevant technical events during operation. Log files can be assessed via the menu bar **Help → Show Log Files**. Log files are intended to support error investigation as well as technical support and other services.

The following warning messages and comments can be logged (see Table 7).

Please note, that validation of peak assignments in GeneMapper™ is critical. Double-assignments (except in CEBPA bZIP INDEL or FLT3 ITD) may invalidate the target region. Unassigned peaks are not evaluated by the Mentype® AMLplus Profiler and may lead to invalidation.
Due to the specific amplification profile of NPM1 (WT and Ins), signal-intense off-ladder peaks ('OL' or '?') not filtered by the analysis method and stutter settings may trigger warning messages and inconclusive results. Please verify in the EPG whether a stutter or rare insertion length, consistent with the same amplification profile, is present.
Spikes, artefacts, or pull-up peaks must be identified by the laboratory professional based on peak shape and intensity, because they may interfere with the analysis depending on device setup. Off-ladder peaks are independently detected by the Mentype® AMLplus Profiler, but artefacts labeled as target peaks may cause false-positive results.

Table 7. Warning messages or comments displayed by the Mentype® AMLplus Profiler and corresponding troubleshooting

Warning message/ comment	Troubleshooting
Refer to troubleshooting instruction.	Sample invalidity probably due to experiment error. Check PCR set up and protocol, if external controls are also invalid. Check pre-analytical workflow in case of sample invalidity only. Validate correct peak assignment in GeneMapper™ if OL warning messages are displayed for the respective targets.

Warning message/ comment	Troubleshooting
Refer to troubleshooting instruction. (if 'OL'/ '?' detected in CEBPA bZIP marker)	A small frameshift INDEL (OL/? of +/- 2 bp from WT) in CEBPA bZIP might be detected. Verify in the EPG if the OL represents a true amplicon or an artefact, characterized by broad and uncommon peak shapes and lower signal intensities. Manual reassignment may be required if an artefact is suspected. If it is a real amplicon, a frameshift CEBPA bZIP INDEL may be detectet that disrupts the coding frame and typically abolishs bZIP funtion. Consider frame status together with other mutational information when evaluating or reanalysis with alternate methods in doubtful cases.
No signal. Refer to troubleshooting instruction.	Sample invalidity. Check PCR set up and protocol, if external controls are also invalid. Check pre-analytical workflow in case of sample invalidity only.
[#] nonspecific peak(s) detected at this locus. Check EPG.	An off-ladder detection can be result of unspecific amplification, a shifted amplicon or an artefact formation. Artefacts like dye blobs are characterized by broader, uncommon peak shapes and lower signal intensities. Please verify within the EPG if real amplicons must be manually assigned to correctly evaluate with the Mentype® AMLplus Profiler.
[AR]/[PMA] calculated from sum of all insertions.	More than one FLT3 ITD was detected in this sample. The AR or PMA represents the result of all ITD mutants. The reported value does not reflect the abundance of individual ITD clones and interpretation should consider the presence of multiple ITD clones.
No WT allele detected. [AR]/[PMA] cannot be determined due to high FLT3 ITD abundance. INDEL length is an estimation referenced to the FLT3 WT of the PC.	The sample may contain a very high allelic burden of FLT3 ITD. Verify in the EPG that no WT allele is detectable or whether the WT peak may be present as an OL peak and requires reassignment. In uncertain cases, confirm the result using an alternative method. The detected ITD length represents an estimate and may slightly differ from the biological insertion length.

Warning message/ comment	Troubleshooting
High signal.	The sample may contain a very high allelic burden of the detected mutation (FLT3 TKD, bZIP INDEL, NPM1, IDH1, or IDH2). This message supports interpretation of mutation abundance during evaluation.
Unexpected high signal. Refer to troubleshooting instruction.	The unexpected high signal of FLT3 TKD (a) may indicate a very high mutation abundance of TKD. Verify in the EPG that no FLT3 TKD (b) is detectable or whether this amplicon may be present as an OL peak and requires reassignment. In uncertain cases, confirm the result using an alternative method.
More than 1 target detected. Refer to troubleshooting instruction.	Two or more mutations within one hotspot region (except FLT ITD) detected are rare and may reflect multiple clones. Verify in the EPG that all assigned peaks represent true amplicons and not artefacts, characterized by broad and uncommon peak shapes and lower signal intensities. Manual reassignment may be required if artefacts are suspected.
Frameshift INDEL.	The detected CEBPA bZIP INDEL disrupts the coding frame and typically abolishs bZIP funtion. Review the EPG to confirm the peak represents a true amplicon. Consider frame status together with other mutational information when evaluating.
In frame INDEL.	The detected CEBPA bZIP INDEL preserves the coding frame and may retain the protein function. Review the EPG to confirm the peak represents a true amplicon. Consider frame status together with other mutational information when evaluating
High signal. Reading-frame status must be confirmed.	The sample may contain a very high allelic burden of the detected CEBPA bZIP mutation. Repeat PCR with diluted sample for precise evaluation of reading frame.
Unexpected INDEL size for NPM1. Refer to troubleshooting instruction.	A rare NPM1 insertion in exon 11 might be detected, characterized by an INDEL length ≠ 4 bp. Verify in the EPG if the OL represents a true amplicon or an artefact, characterized by broad and uncommon peak shapes and lower signal intensities. Manual reassignment may be required if an artefact is suspected. In uncertain cases, confirm the result using an alternative method.

Warning message/ comment	Troubleshooting
Known dye-related artefact detected. Check EPG.	A known artefact has been detected within the IDH1 R132 panel. This artefact is related to the assay design and a specific run behavior on certain polymer types, but does not interfere with specific targets. In uncertain cases please contact BIOTYPE's customer support.
Possible false positive detection of [R132S]/[R172W]. Verify result.	Bordeline detection of IDH1 R132S or IDH2 R172W with higher risk of false-positivity. Confirmation with alternate method is recommended for this target. 2 or more IDH1 or IDH2 variants in one sample are a rare event, but may reflect multiple subclones. Verify in the EPG if the assigned peak represents a true amplicon (called as R132S/R172W) or an artefact, characterized by broad and uncommon peak shapes and lower signal intensities. Manual reassignment may be required if an artefact is suspected. In uncertain cases, confirm the result using an alternative method. This warning message is displayed if ratio IDH1 R132G/R132S ≤ 6 or ratio IDH2 R172K/R172W ≤ 10 due to sporadic, non-specific co-amplifcation.
Possible false negative detection of [R132S]/[R172W]. Verify result.	Bordeline detection of IDH1 R132S or IDH2 R172W with higher risk of false-negativity. Confirmation with alternate method is recommended for this target. 2 or more IDH1 or IDH2 variants in one sample are a rare event, but may reflect multiple subclones. Verify in the EPG if the assigned peak represents a true amplicon (called as R132S/R172W) or an artefact, characterized by broad and uncommon peak shapes and lower signal intensities. Manual reassignment may be required if an artefact is suspected. In uncertain cases, confirm the result using an alternative method. This warning message is displayed if ratio IDH1 R132G/R132S > 6 or ratio IDH2 R172K/R172W > 10 due to sporadic, non-specific co-amplification, considered as background noise, and signal intense real-positive IDH1 R132G or IDH2 R172K detection

Warning message/ comment	Troubleshooting
Target not found. Please check EPG and verify peak assignment.	PC, NTC or AL is invalid due to an incomplete profile or samples might be invalid due to missing IC. Verify in the EPG that no target is detectable or whether this target may be present as an OL peak and requires reassignment. Repeat the PCR with well-mixed reagents. Consider errors in kit storage or handling. Or repeat the fragment length analysis and include a well-mixed allelic ladder
Unexpected target detected.	NTC, PC or AL invalid, because several unexpected targets have been detected. Contamination might be the cause of the error. Repeat the run in a clean working environment if necessary. False peak assignment due to dye blobs or other artefacts could also be the cause of error, hereby EPG validation is recommended.
Data manipulation. Please use correct analysis method. Peak height [height] is below the limit-of-blank threshold 400 in line [line].	Warning: manipulated TXT input to the Mentype® AMLplus Profiler. Peak heights below the assay cut-off of 400 RFU have been detected, which invalidates the run unless alternative procedures have been validated under the customer's own responsibility. Ensure that only the approved template files are used for GeneMapper™ analysis (Analysis Methods, Bin, Panels, Size Standard, Stutter).
The file has no content./ Could not read the file./ Invalid peak area/heigth/size/sample name/dye [value] in line [line]./ Unknown allele/marker [value] in line [line]./ The target for allele '[allele]' was not found./ The column [column] was not found in line [line]./ The peak size [size] is duplicated.	Incorrect file format or incorrect content of TXT input for profiler. Please make sure to use the TXT data export of the GeneMapper™ and to define the layout as defined within the recommended Plot settings.

NOTE

If other warning messages occur, please forward the log file to Customer Support for further investigation: support@biotype.de

NOTE

Please also refer to the handbook of the Mentype® AMLplus Mutation Analysis Kit at www.biotype.de/en/ifus.

Cybersecurity

This chapter describes the cybersecurity characteristics of the Mentype® AMLplus Profiler and the measures required to operate it securely. The software is a locally installed, standalone application that runs fully offline. It requires no network connection, server, or database. Its security therefore depends on the protection of the host computer and on the integrity of the imported and exported files.

Secure installation and configuration

The software must be installed by qualified or authorized personnel using only the original installation package provided by BIOTYPE. The installer and the application are digitally signed; before installation, verify that the publisher is identified as BIOTYPE GmbH and that the operating system reports a valid signature. Do not install packages whose signature cannot be verified.

Data privacy

The Mentype® AMLplus Profiler is designed as a stateless, real-time application and does not store data permanently. Nonetheless, access should be restricted to authorized personnel only. This also applies to exported data. Access restrictions are ensured exclusively through the user account and login credentials of the host PC.

Data integrity and file traceability

The Mentype® AMLplus Profiler software imports experimental data in the form of GeneMapper™ TXT output files. TXT files are not inherently protected against modification after export from the originating system. Any alteration of file content may compromise the integrity and reliability of the analytical results. To support data integrity verification and traceability, the software automatically calculates and records a cryptographic hash value of each imported file.

Upon import of a GeneMapper™ TXT file, the software automatically:

- Reads the complete file content
- Calculates a SHA-256 (Secure Hash Algorithm 256-bit) hash value
- Associates the calculated hash value with the analysis session

The SHA-256 hash value is a unique digital fingerprint of the imported file. Any modification of the file content, including a single character change, results in a different hash value. The hash calculation is performed automatically and does not require user interaction. The calculated SHA-256 hash value is displayed in the software status bar after successful file import and included in the generated pdf report. Thereby, the retrospective traceability of the exact data file used for analysis is ensured.

User responsibilities

The software does not prevent the import of modified or manipulated TXT files. It is the responsibility of the user and the laboratory quality management system to ensure the secure storage of original GeneMapper™ output files, the controlled access to raw data files, the verification of file integrity where required and the appropriate documentation in accordance with laboratory procedures. Where data integrity verification is required, the SHA-256 hash value displayed in the status bar or printed in the PDF report shall be compared to a newly calculated hash value of the source file using an independent tool. If hash values do not match, the analysis results shall not be used for reporting. This functionality supports, but does not replace, laboratory quality assurance procedures.

Maintenance and updates

Regularly update Mentype® AMLplus Profiler to the latest version to protect against vulnerabilities. Apply security patches and updates as soon as they are available. Keep the host operating system and antivirus software up to date

Log files

The software automatically generates log files documenting relevant technical events during operation. Log files can be accessed via the menu bar **Help → Show Log Files.** Log files are intended to support error investigation, technical support and service activities.

About dialog

The Mentype® AMLplus Profiler software provides an About dialog containing device and regulatory information. The About dialog can be accessed via **Help → About…**

The About dialog includes information on software version number, manufacturer information, regulatory status, information on incorporated third-party software components (SOUP – Software of Unknown Provenance) and Bill of Materials (BOM), including relevant software components and version identifiers.

Incident response

If suspicious behavior is observed, users should consult their IT administrator or IT security officer immediately. Form an incident response team capable of responding to cybersecurity threats. Develop a comprehensive disaster recovery plan to restore functionality and data in the event of a cybersecurity incident.

Decommissioning

When the software is uninstalled or the host computer is retired, securely delete all locally stored input files, exported reports, and log files that contain sample data, in accordance with the laboratory's data protection procedures. If the computer is transferred or disposed of, perform a complete wipe of the storage media.

Training and awareness

Conduct regular training sessions for all users to understand the cybersecurity policies and procedures related to Mentype® AMLplus Profiler.

Implement ongoing awareness campaigns to keep security at the forefront of operations.

References

[1] The Cancer Genome Atlas Research Network: Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. N Engl J Med VOL. 368 NO. 22. 2013; 368:2059-2074. DOI: 10.1056/NEJMoa1301689

Technical assistance

For technical advice, please contact our Customer Support Team:

e-mail: support@biotype.de

phone: +49 (0)351 8838 400

Limitations of use

- The procedures in this handbook must be followed, as described. Any deviations may result in error messages.
- The Mentype® AMLplus Profiler is a software solution for data evaluation based exclusively on TXT output files generated by GeneMapper™ analyses of the Mentype® AMLplus Mutation Analysis Kit. Data evaluation is dependent on prior raw data analysis using the GeneMapper™ software.
- Use of this product is limited to professional laboratory users trained on molecular-genetic techniques, multiplex PCR, and the handling of Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division).
- Results must be interpreted by professionals in context with results of other relevant methods.
- Interpretation of results must account for the possibility of false negative and false positive results
- Foreseeable misuse is mitigated by software restrictions and corresponding warning messages. Any manipulation of raw data is the responsibility of the user.

Ordering information

Direct your orders via email to sales@biotype.de.

Product	Packaging size	Order number
Mentype® AMLplus Mutation Analysis Kit	25 reactions	BT00005
	100 reactions	BT00006
Matrix Standard BT5 multi	1 x 25µL	45-15100-0025
	2 x 25µL	45-15100-0050

Trademark and disclaimers

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Other trademarks: GeneMapper™, SeqStudio™ and Applied Biosystems® (Applied Biosystems LLC group). The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche). This product incorporates proprietary technology that is patented and/or patent pending.

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Explanation of Symbols

	Manufacturer
	Batch code/ software version
	Reference to eIFU
	Catalogue number
RUO	For research use only

Further marking used in this Instruction for Use:

	Useful tips
	Attention, be sure to follow this notice!
blue underlined text	Links leading to external content like homepages, e-mail adresses
black underlined text	Cross-links in the document for easy navigation
cursive or **bold** text	Fields which are to be clicked in a software

Appendix

Validity criteria for external PCR controls

NOTE

If the sample name of the Positive Control contains "**PC**", the No-Template Control contains "**NTC**", and the Allelic Ladder contains "**AL**". The Mentype® AMLplus Profiler software can automatically identify the controls. In this case, no further reassignment is required during the data evaluation procedure.

- **Mentype® AMLplus Allelic Ladder (AL)**

All required peaks in the allelic ladder are present with ≥ 400 RFU. The complete profile must be detected (see Table 8). Accurate assignment requires a passing CGQ (composite genotyping quality) assessment.

Table 8. Control peaks in Mentype® AMLplus Allelic Ladder
Fragment lengths [bp] are approximate and may vary due to environmental factors

Panel	Allelic ladder peak	Length [bp]		
		POP-1™	POP-4™	POP-7™
Blue	FLT3 TKD (a)	80	86	123
	FLT3 TKD (b)	98	117	150
	CEBPA bZIP Del	364	363	363
	CEBPA bZIP WT	382	381	381
	CEBPA bZIP Ins	434	433	433
Green	IDH1 R132H	112	112	112
	IDH1 R132C	115	116	115
	IDH1 R132G	119	120	120
	IDH1 R132S	121	122	122
	IDH1 R132L	124	125	125
	IDH2 R172K	146	148	147
	IDH2 R172W	154	156	155
	NPM1 WT	187	187	188
	NPM1 Ins	191	191	192

Panel	Allelic ladder peak	Length [bp]		
		POP-1™	POP-4 ™	POP-7 ™
	IDH2 R140Q	244	245	244
	IDH2 R140L	251	252	251
	IDH2 R140W	253	254	254
Yellow	AC	172	174	173
	FLT3 ITD WT	313	314	313
	FLT3 ITD	316	317	316

- **Mentype® AMLplus Positive Control (PC)**

All required peaks in the positive control are present with ≥ 400 RFU. The complete profile must be detected (see Table 9). The Mentype® AMLplus Positive Control also functions as a negative control (wildtype) for the FLT3 TKD target, which is required for the qualitative assessment of FLT3 TKD status in all samples.

Table 9. Control peaks in Mentype® AMLplus Positive Control

Panel	Positive control peak
Blue	▪ FLT3 TKD (a) ▪ FLT3 TKD (b) ▪ CEBPA bZIP WT
Green	▪ IDH1 R132H ▪ IDH2 R172K ▪ NPM1 WT ▪ NPM1 Ins ▪ IDH2 R140Q
Yellow	▪ AC ▪ FLT3 ITD WT

NOTE

Using the Mentype® AMLplus Positive Control, pull-up peaks may occasionally be observed for certain wildtype alleles, such as NPM1 WT or FLT3 ITD WT. The positions of all wildtype targets across the different channels have been intentionally selected to avoid interference with other measuring ranges or panels. Off-marker-range (OMR) peaks or pull-up peaks appearing between panels are expected and can be disregarded.

NOTE

For FLT3 TKD (a) and FLT3 TKD (b) the analytes are probe hydrolysis products instead of amplicons. Thus peaks are expected to appear slightly broader compared with the other fragments.

- **No-template Control NTC**

No peaks ≥ 400 RFU shall be detected, except the Amplification Control AC, that is a template-independent internal control.

For the target FLT3 TKD competing probes are analyzed. Therefore, it might happen that especially the FLT3 TKD (a) peak exceeds this cut-off. If no other alleles are detected, this does not indicate contamination.

NOTE

Artefacts like small dye blobs may occur more prominently within the NTC. Because of the broad peak base, abnormal peak shape and no peak assignment, a differentiation from amplicons is possible.

Validity criteria for samples

Table 10. Internal Controls (IC)

Type of internal control	Definition
Amplification Control (AC)	The Amplification Control (AC) is a template-independent internal control present in all sample types (controls and samples) within the yellow channel, that indicates proper PCR setup as well as kit storage and performance.
Wildtype controls used for validation • NPM1 WT • CEBPA bZIP WT	Wildtype ICs are used as a template-dependent internal control, present in all samples as well as the Mentype® AMLplus Positive Control. NPM1 WT (green channel) and CEBPA bZIP WT (blue channel) are indicating proper sample quality.
Other wildtype controls • FLT3 ITD WT	FLT3 ITD WT is defined as internal wildtype control. However, the internal wildtype control may be absent in samples with a high FLT3 ITD mutational burden and does not lead to invalidation if at least one ITD allele is detected. FLT3 wildtype is detected within the yellow channel. A minimum of ≥ 400 RFU must be achieved, either for the FLT3 wildtype or the FLT3 ITD allele.

A minimum of ≥ 400 RFU must be achieved for all internal controls.

Sample analysis

Once run and sample validity have been confirmed, the specific targets within the sample can be analyzed. All targets (except target FLT3 TKD) detected above the assay cut-off ≥ 400 RFU are considered positive.

For the **FLT3 TKD** target, two peaks are consistently detected in the electropherogram: FLT3 TKD (a) and FLT3 TKD (b), representing two competing probes targeting the same genetic region. In the presence of a

mutation, the upstream probe FLT3 TKD (a) typically shows the dominant signal intensity, whereas the downstream probe FLT3 TKD (b) predominates in wildtype samples. For qualitative assessment of the target region, the following calculations are used by the Mentype® AMLplus Profiler:

(1) $R = \frac{AUC_{FLT3\ TKD\ (a)}}{AUC_{FLT3\ TKD\ (b)}}$

(2) $\Delta R = R_{sample} - R_{PC}$

(3) $R > 0.04 \Longrightarrow \text{FLT3 TKD positive}$

AUC… area under the curve
R… Ratio

FLT3 ITD is primarily assessed qualitatively, determining the presence or absence of one or more ITD alleles in the sample. Additionally, a semi-quantitative assessment of the mutational burden can be performed by calculating the allelic ratio (AR) or the proportion of mutant alleles (PMA).

The **AR** is calculated as follows:

$$\text{AR} = \frac{\sum(AUC\ ITD\ alleles)}{AUC\ wildtype\ allele}$$

The **PMA** is calculated as follows:

$$\text{PMA} = \frac{\sum(AUC\ ITD\ alleles)}{(AUC\ wildtype\ allele) + \sum(AUC\ ITD\ alleles)}$$

CEBPA bZIP in-frame INDELs are primarily assessed qualitatively, determining the presence or absence of an INDEL within the bZIP domain of the CEBPA gene. The reading frame of each detected INDEL is additionally determined based on the size of the insertion or deletion relative to the codon structure.

Indels are classified as follows:

- **In-frame INDELs:** insertion or deletion of a number of nucleotides divisible by 3, preserving the reading frame and resulting in the addition or loss of complete amino acids.

- **Frameshift INDELs:** insertion or deletion of a number of nucleotides not divisible by 3, disrupting the reading frame and typically leading to a premature stop codon and a truncated or non-functional protein.

The reading frame classification can be calculated in three steps:

First CEBPA bZIP INDEL size is calculated by subtraction of INDEL length from the WT control size and then corrected by a correction factor (CF) derived from the Allelic Ladder (AL).

(1) $CEBPA\ bZIP\ INDEL\ size = CF * (size_{INDEL} - size_{WT})$

(2) $CF = 0.5 * \frac{AL\ size_{INS} - AL\ size_{WT}}{51\ bp} + \frac{AL\ size_{WT} - AL\ size_{Del}}{18\ bp})$

(3) $\frac{CEBPA\ bZIP\ INDEL\ size}{3} \in \mathbb{Z} \Longrightarrow \text{in frame}$

An additional **plausibility** check shall be performed for each sample. For instance, the detection of three or more mutations within one target region is uncommon and may reflect the presence of multiple clones; these findings should be confirmed by visual review of the electropherogram or, in uncertain cases, by alternative methods. In case of any uncertainties refer to the handbook of the Mentype® AMLplus Mutation Analysis Kit or contact BIOTYPE's customer support via support@biotype.de.

NOTE

If multiple target detections exceed the cut-off or if you encounter any uncertainties, please check the troubleshooting section or reach out to customer support at support@biotype.de

NOTE

Analyzing samples, pull-up peaks may occasionally be observed for certain wild-type alleles, such as NPM1 WT or FLT3 ITD WT. The positions of all wildtype targets across the different channels have been intentionally selected to avoid interference with other measuring ranges or panels. Off-marker-range (OMR) peaks or pull-up peaks appearing between panels are expected and can be disregarded.

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