Mentype® **AMLplus**

Mutation Analysis Kit

Handbook

RUO For research use only. Not for use in diagnostic procedures.

APMHB01v1en
17.07.2026

REF BT00005
BT00006

LOT Lot number

BIOTYPE GmbH
Moritzburger Weg 67
01109 DRESDEN
GERMANY
Website: www.biotype.de
Email: support@biotype.de
Ordering: sales@biotype.de

BIOTYPE

Notice of Change

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

Document code	Changes	Date
APMHB01v1en	Initial version	17.07.2026

For any further questions, please contact us:

at +49 351 8838 400 or

support@biotype.de

Content

Product description

The Mentype® AMLplus Mutation Analysis Kit is a manual, PCR-based multiplex assay for the qualitative detection of mutation variants in multiple genomic target regions that have been described in samples associated with Acute Myeloid Leukemia (AML).

Genomic DNA isolated from peripheral blood or bone marrow may be used as input material for multiplex PCR, in which five target genes are amplified simultaneously (see Table 1). The resulting fluorescence-labelled PCR products are analyzed by fragment length analysis on a Genetic Analyzer (Thermo Fisher Scientific). A secondary software tool, the Mentype® AMLplus Profiler, provided with the kit supports the standardized evaluation of the generated data. Interpretation of results and any subsequent conclusions are the responsibility of the qualified laboratory research professional.

All target regions are analyzed qualitatively to determine the presence or absence of specific sequence variants. In addition, the assay enables a semi-quantitative assessment of FLT3 ITD related signal patterns by calculating the ratio of variant to reference alleles, allowing estimation of the relative abundance of the respective variant allele within the analyzed sample.

Table 1. Mutant variants detectable with Mentype® AMLplus Mutation Analysis Kit.
#variant assignment in accordance with output of Mentype® AMLplus Profiler

Gene	Target#	Alteration
NPM1	Wildtype (WT)	Wildtype of the exon 11† hotspot region
	Insertion* (Ins)	4bp insertions in exon 11† leading to a premature stop codon. These alterations include: - Type A - Type B - Type C - Type D - And rarer 4 bp insertions As well as rare types characterized by a variable bp insertions.

Gene	Target#	Alteration
FLT3	ITD Wildtype (WT)	Wildtype detection of the juxtamembrane and tyrosine kinase domain 1 (TKD1)
	ITD	3-237 bp Internal Tandem Duplications in the juxtamembrane domain and TKD1
	TKD	Selected mutations* in tyrosine-kinase domain 2 on codons D835 and I836 including but not limited to: - D835Y - D835H - I836del - D835V - D835N - D835G - D835S - D835del - D835A - D835E - D835F
IDH1	R132	R132H
		R132C
		R132G
		R132S
		R132L
IDH2	R140	R140Q
		R140W
		R140L
	R172	R172K
		R172W
CEBPA	bZIP Wildtype (WT)	Wildtype of the bZIP domain
	bZIP Insertion	3-170 bp Insertions
	bZIP Deletion	3-18 bp Deletions

† NPM1 NM_002520.7 Exon 11 was formerly named Exon12, this refers to the last coding exon
*variants cannot be discriminated

Scientific background

Acute Myeloid Leukemia (AML) is a biologically heterogeneous disorder of the hematopoietic system characterized 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

Table 2. Kit content of Mentype® AMLplus Mutation Analysis Kit

Kit component	Cap color	Volume per packaging size	
		25 reactions	100 reactions
Nuclease-Free Water	Light blue	1.5 mL	1.5 mL
PCR Buffer 12	Black	315 µL	1250 µL
Mentype® AMLplus Primer Mix	Yellow	63 µL	250 µL
Multi Taq 2 DNA Polymerase	White	15 µL	60 µL
Mentype® AMLplus Positive Control	White	25 µL	25 µL
DNA Size Standard 550 (BTO)	Orange	13 µL	50 µL
Mentype® AMLplus Allelic Ladder	Green	30 µL	30 µL
Mentype® AMLplus Profiler*		download	

*The software Mentype® AMLplus Profiler can be downloaded at www.biotype.de/en/software
The **password** required for the download is included in the kit upon delivery.

An overview of the component batch numbers is provided on the label located on the inside of the box flap.

NOTE

Please note that the packaging size describes the number of tests **without** considering the number of required controls or the required excess for pipetting. We recommend using the following size for the corresponding throughput:

- < 5 samples per PCR run: 25 reaction packaging size
- > 5 samples per PCR run: 100 reaction packaging size

Description of components

Nuclease-Free Water: PCR grade water, used in the PCR set-up and as no template control (NTC).

PCR Buffer 12: PCR buffer containing dNTPs, $MgCl_2$ and DMSO. The PCR buffer is optimized to promote enzyme activity for the PCR.

Mentype® AMLplus Primer Mix: multiplex oligonucleotide primer mix containing labeled primer or probes (label: 6-FAM™, BTG, BTY) and unlabeled primers.

Multi Taq 2 DNA Polymerase: hot start Taq DNA polymerase, 2.5 U/µL.

Mentype® AMLplus Positive Control: double stranded artificial DNA mixture. It is used as a qualitative, external PCR control, which represents the targets FLT3 ITD WT, CEBPA bZIP WT, IDH1 R132H, IDH2 R172K, IDH2 R140Q, NPM1 WT, NPM1 Ins and covers all 3 detection channels.

In addition, the Mentype® AMLplus Positive Control serves as the negative control (wildtype) for the FLT3 TKD target and is used for the FLT3 TKD evaluation procedure as described in chapter General information on data analysis.

NOTE

The Mentype® AMLplus Positive Control is not critical for the laboratory professional, as it consists of individual DNA molecules that have been purified, are non-hazardous and have no active biological functions. It contains no living cells or pathogenic organisms that could pose a direct threat.

DNA Size Standard 550 (BTO): mixture of fluorophore-labeled PCR fragments with defined fragment lengths between 60 bp and 550 bp. The component is added to each PCR product before the fragment length analysis and is used for a size regression to exactly determine the fragment length of the PCR products.

Mentype® AMLplus Allelic Ladder: a mixture of fluorophore-labeled PCR fragments representing certain detectable alleles of the Mentype® AMLplus Mutation Analysis Kit. It includes 5 fragments labeled with 6-FAM™, 12 fragments labeled with BTG and 3 fragments labeled with BTY and is used as a genotyping reference for the exact allele identification.

Mentype® AMLplus Profiler: a software for data analysis and evaluation as an integral, non-standalone component of the kit. It can be downloaded at www.biotype.de/en/software with a password that is provided within the kit upon delivery.

NOTE

For detailed information on operating the Mentype® AMLplus Profiler, please refer to the corresponding software handbook.

Reagent storage and handling

The kit is shipped on dry ice. The components of the kit should arrive frozen, except the Multi Taq 2 DNA Polymerase, that is stored in a buffer preventing freezing of the reagent. Furthermore, PCR Buffer 12 may occasionally not freeze. This does not affect performance.

Please check the completeness of the kit upon receipt. Do not use kits that have been thawed upon arrival. If any components other than the above

mentioned are not frozen, or if tubes or the packaging have been compromised during the shipment, the performance cannot be guaranteed.

Store all components between -25 °C to -15 °C and protected from light. Especially the Mentype® AMLplus Primer Mix, DNA Size Standard 550 (BTO) and Mentype® AMLplus Allelic Ladder must be stored protected from light.

To prevent contamination, we recommend that pre-amplification components (DNA samples, the Mentype® AMLplus Positive Control) and the post-amplification components (DNA Size Standard 550 (BTO) and Mentype® AMLplus Allelic Ladder) are used separately from PCR reagents (Nuclease-Free Water, Multi Taq 2 DNA Polymerase, PCR Buffer 12 and Mentype® AMLplus Primer Mix).

The kit will expire according to the information on the kit box label. Do not exceed a maximum of 20 freeze-thaw cycles.

Material and devices required but not provided

General laboratory equipment

- Desktop centrifuge with a rotor for 2 mL and 200 µL reaction tubes
- Centrifuge with a rotor for microtiter plates for 96 well reaction plates
- Vortex mixer
- Calibrated adjustable pipettes with disposal aerosol tight filter tips
- Appropriate 200 µL 96-well reaction plates or 200 µL reaction tubes with corresponding closing material, PCR grade
- Suitable racks for 2 mL and 200 µL reaction tubes
- Cooling rack suitable for 2 mL tubes
- Disposable powder-free gloves
- Qubit™ Fluorometer

NOTE

All material used for PCR shall have appropriate quality (DNA free and for molecular biology). Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturers' instructions and recommendations.

Reagents, kits and consumables

Table 3. Reagents required, but not provided
Please note that some reagents and consumables are used in specific combinations and are not all required for every run

Reagent	Supplier	Order number
Matrix Standard BT5 multi (25 µL)	BIOTYPE GmbH	45-15100-0025
Matrix Standard BT5 multi (2 x 25 µL)	BIOTYPE GmbH	45-15100-0050
QIAamp® DSP DNA Blood Mini Kit	QIAGEN	61104
QIAamp® DNA Mini Kit	QIAGEN	51304
NucleoSpin® DX Blood	Macherey-Nagel	740899.50
Qubit™ dsDNA BR Assay Kit	Thermo Fisher Scientific	Q32850
Maxwell® RSC Whole Blood DNA Kit	Promega	AS1520
Hi-Di™ Formamide, 25 mL	Thermo Fisher Scientific	4311320
POP-4™ Polymer for 3500/3500xL Genetic Analyzers (96 samples)	Thermo Fisher Scientific	A26070
POP-4™ Polymer for 3500/3500xL Genetic Analyzers (384 samples)	Thermo Fisher Scientific	4393715
POP-7™ Polymer for 3500/3500xL Genetic Analyzers (96 samples)	Thermo Fisher Scientific	A26073
POP-7™ Polymer for 3500/3500xL Genetic Analyzers (384 samples)	Thermo Fisher Scientific	4393708
Anode Buffer Container (ABC) 3500 Series	Thermo Fisher Scientific	4393927
Cathode Buffer Container (CBC) 3500 Series	Thermo Fisher Scientific	4408256
3500 Genetic Analyzer 8-Capillary Array 36 cm	Thermo Fisher Scientific	4404683
3500 Genetic Analyzer 8-Capillary Array 50 cm	Thermo Fisher Scientific	4404685
SeqStudio™ Cartridge	Thermo Fisher Scientific	A33671 A41331
SeqStudio™ Cathode Buffer	Thermo Fisher Scientific	A33401

Instruments and software

Next to the column-based extraction methods as described in chapter DNA extraction, the following automation can be used for DNA extraction:

- Maxwell® RSC Instrument (cat. no.: AS4500, Promega)
- QIAcube Connect (cat.no.: 9002864, Qiagen)

The Mentype® AMLplus PCR Amplification Kit was verified to be used with the following PCR cyclers:

- ProFlex PCR System (cat. no.: 4484073 (3 x 32 Well sample block), 4484075 (96-Well sample block), Thermo Fisher Scientific)
- GeneAmp® PCR System 9700 Ag (discontinued, Thermo Fisher Scientific)
- Mastercycler® Nexus Gradient (cat. no.: 6331000017, Eppendorf AG)

The application of PCR cyclers other than the before stated, must be verified by the user. The following specifications must be fulfilled:

- Heated lid
- Block suitable for 200 µL reaction plates / tubes
- Ramping adjustable to 1 °C/s

The Mentype® AMLplus Mutation Analysis Kit was verified to be used with the following instrument and settings:

- Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific), software version 4.0.1
  - POP-4™ Polymer for 3500/3500xL Genetic Analyzer
  - POP-7™ Polymer for 3500/3500xL Genetic Analyzer
  - 3500 Genetic Analyzer 8-Capillary Array 36 cm
  - 3500 Genetic Analyzer 8-Capillary Array 50 cm
- SeqStudio™ Genetic Analyzer (Thermo Fisher Scientifc), software version 1.2.4

The data analysis was performed with the software:

- Initial raw data analysis by using GeneMapper™ ID-X v1.6 or GeneMapper™ Software 6.1 (Thermo Fisher Scientific), using one of the product specific templates (see also Table 9 in chapter Requirements for data analysis)

A manual evaluation of the fsa-files or any results given with the software for data collection without the software described for data analysis and evaluation is not validated.

Specimen and test samples

The following human specimens have been validated with the Mentype® AMLplus Mutation Analysis Kit:

- Peripheral venous whole blood samples (EDTA, citrate), transported and stored at 22-25 °C for up to 24 h or at 2-6 °C for up to 72 h
- Bone marrow samples (EDTA), transported and stored at 4 °C up to 24 h.

The obtained undiluted gDNA shall be stored frozen.

NOTE

Please ensure that the anticoagulant used for blood collection is compatible with the DNA isolation kit's manufacturer's instruction.

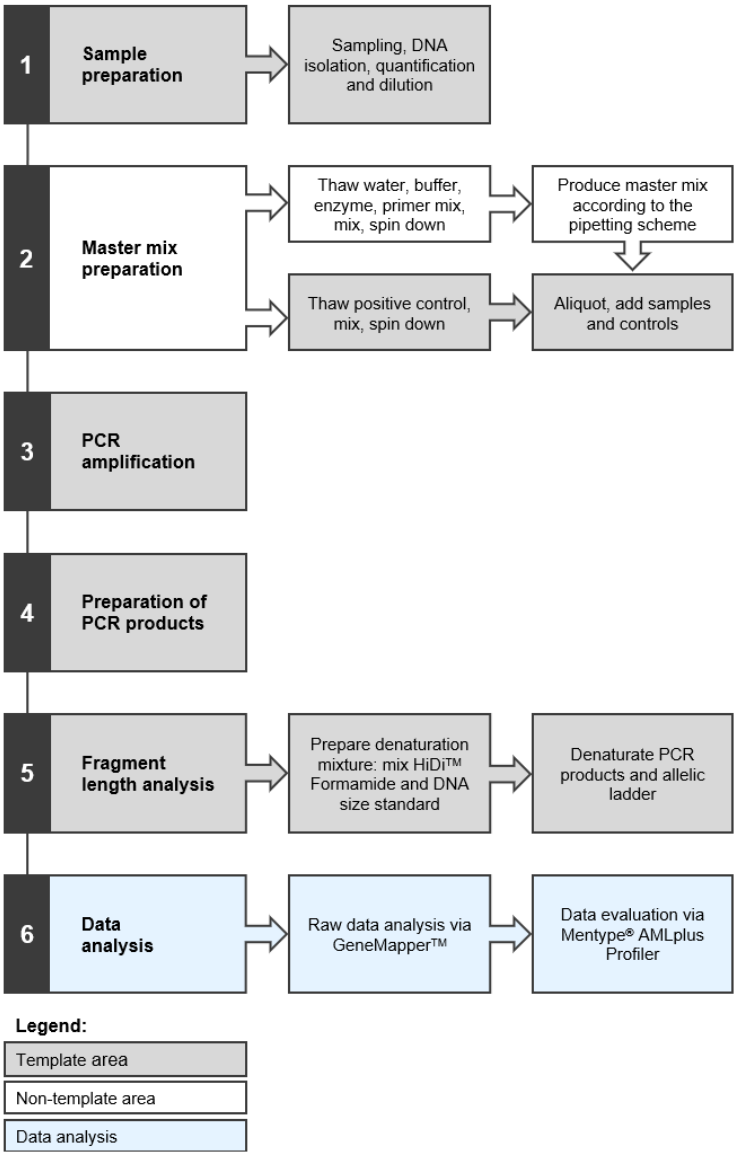
Warnings and precautions

- Read the handbook carefully before using the product.
- Read the safety data sheets (SDS) and non-hazardous statements (NHS) for all BIOTYPE products, which are available on request or via (www.biotype.de/en/sicherheitsdatenblatter). For products that do not require a SDS as they do not contain an SVHC or are subject to other restrictions of Regulation 1272/2008 (CLP), BIOTYPE provides the SDS upon request.
- Please contact the manufacturers of the materials and reagents required, but not provided for copies of the SDS for any additionally needed reagents.
- Kit components of different kit lots must not be mixed.
- Aliquoting the kit components into other reaction vessels is not permitted.
- Before the first use, check the product and its components for:

  - Integrity
  - Completeness with respect to number, type and filling (see chapter Materials provided)
  - Correct labelling
  - Frozenness upon arrival (except the Multi Taq 2 DNA Polymerase)
- Specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures.
- Do not use a kit that has passed its expiration date.
- Discard sample and assay waste according to your local safety and disposal regulations.

Procedure

Overview of experimental workflow



Sample preparation

Raw sample requirements

The specimen for the Mentype® AMLplus Mutation Analysis Kit is defined as genomic DNA (gDNA) extracted from peripheral venous whole blood or bone marrow aspirates collected from humans.

NOTE

A long storage of the raw sample material might lead to a fragmentation of the genetic material and therefore, lead to an insufficient quality of the material. This can worsen the analysis result, e. g. through decreased signals or invalid internal controls.

Blood

The handling of the raw sample material peripheral venous whole blood should follow the recommendation of the Clinical and Laboratory Standards Institute (CLSI) guideline MM05–A2 (2nd edition): blood can be stored at room temperature (22 °C to 25 °C) for up to 24 hours, or at 2 °C to 6 °C for 72 hours or more. Additionally, it is recommended that the anticoagulants used for whole blood collection are EDTA or citrate in relation to the workflow described.

Bone marrow

The handling of bone marrow aspirates should follow the recommendation of the CLSI guideline MM05–A2 (2nd edition), where it is stated that anticoagulated (EDTA or citrate) bone marrow aspirates should be transported and stored at 4 °C for up to 24 h.

DNA extraction

Perform the gDNA extraction and purification from peripheral venous whole blood or bone marrow aspirates with one of the following kits:

- QIAamp® DSP DNA Blood Mini Kit
- NucleoSpin® DX Blood
- QIAamp® DSP DNA Mini Kit
- Maxwell® RSC Whole Blood DNA Kit

Please follow the manufacturer's manuals and recommendations for DNA extraction

NOTE

Blood contamination can be visually detected in the PCR reaction or in the isolated gDNA by an orange color shift. If a color change is observed, we recommend repeating the gDNA isolation to avoid any potential interference.

NOTE

Make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of PCR.

DNA quantification and dilution

Quantify the DNA concentration by fluorescence spectroscopy using the Qubit™ Fluorometer and the Qubit dsDNA BR Kit.

For use with Mentype® AMLplus Mutation Analysis Kit, dilute the gDNA samples to an optimal concentration of 20 ng/µL. Prepare the dilution freshly before usage. Use nuclease-free water as diluent.

NOTE

The total **optimum input is 20 ng gDNA** per reaction under standard conditions for highest sensitivity.

DNA storage

DNA should be stored undiluted at -25 °C to -15 °C for long term storage or according to the DNA isolation kit's manufacturer's information.

Master mix setup

Use the following components from the Mentype® AMLplus Mutation Analysis Kit for the master mix setup:

- Nuclease-Free Water (light blue cap)
- PCR Buffer 12 (black cap)
- Mentype® AMLplus Primer Mix (yellow cap)
- Multi Taq 2 DNA Polymerase (white cap)

All frozen components should be thawed at room temperature (22 °C to 25 °C, ca. 30 min, protected from light) and homogenized by gentle vortexing. After this, briefly centrifuge the reagents (approx. 10 s). To uphold the principles of good laboratory practice, it is advisable to keep the Multi Taq 2 DNA Polymerase in a cooled environment as long as possible (e. g. cooling rack) prior to the master mix setup.

NOTE

Mix the Multi Taq 2 DNA Polymerase by flicking for longer stability – **do not vortex the enzyme**.

Prepare the PCR master mix according to Table 4 in an appropriately sized microcentrifuge tube for the total number of samples to be tested in a dedicated clean area. Include at least one PC and one NTC into your calculation.

NOTE

As a rule of thumb, if you are testing fewer than 10 samples, use enough master mix for one extra sample. If you are testing 10 or more samples, use an excess reagent master mix volume of +10 %.

Table 4. PCR master mix reaction setup
#… reactions

	Volume		
Component	**#1**	**#5**	**#10**
Nuclease-Free Water*	8.4 µL	42 µL	84 µL
PCR Buffer 12	12.5 µL	62.5 µL	125 µL
Mentype® AMLplus Primer Mix	2.5 µL	12.5 µL	25.0 µL
Multi Taq 2 DNA Polymerase	0.6 µL	3.0 µL	6.0 µL
DNA template or control sample	1.0 µL*	5 x 1.0 µL*	10 x 1.0 µL*
Total volume	**25.0 µL**	**125.0 µL**	**250.0 µL**

*… The template input can be increased, for this the volume of Nuclease-Free Water must be adjusted.

Mix the master mix by gently vortexing, then briefly centrifuge.

Aliquot 24.0 µL of the PCR master mix in prepared 200 µL PCR tubes and briefly centrifuge the closed tubes.

Addition of samples and controls

Add 1.0 µL of the following sample types to the prepared PCR tubes containing PCR master mix.

NTC: add 1.0 µL of Nuclease-Free Water instead of a sample.

PC: add 1.0 µL of the **undiluted** Mentype® AMLplus Positive Control instead of a sample.

Sample: add 1.0 µL of a 20 ng/µL concentrated gDNA sample.

NOTE

First, prepare the NTC to avoid contamination of the control. Prepare the PC last to avoid cross contamination of the samples.

NOTE

Use one positive control **PC** and one no template control **NTC** per PCR run. Otherwise, the experiment cannot be validated.

Close all PCR tubes, gently vortex and spin down.

PCR amplification

NOTE

Using calibrated and well-maintained PCR cyclers is essential, as deviations can negatively affect the amplification of sensitive targets.

Use the following PCR protocol.

Table 5. PCR protocol

Temperature	Time	Cycle No.	Ramping
96 °C	4 min	1	
96 °C	5 s		
60 °C	45 s	26	1°C/s
72 °C	45 s		
68 °C	60 min	1	1°C/s
10 °C	∞	hold	

NOTE

If thermal cyclers with adjustable heating and cooling rates are used, **ramping shall be adjusted to 1 °C/s** in order to provide an optimal kit balance.

NOTE

For basic information regarding the setup, programming and maintenance of the different PCR instruments, please refer to the user manual of the respective instrument.

Capillary gel electrophoresis

Preparation of PCR products

After completion of the PCR, remove the samples from the cycler and centrifuge briefly

NOTE

After completion of the PCR, the PCR products can be stored for 1 week at 2 °C to 8 °C or long-term at -25 °C to -15 °C protected from light. Storage of PCR products prepared in Hi-Di Formamide or reinjection is not recommended.

Thaw, mix and centrifuge the reagents:

- Hi-Di™ Formamide (not included in the kit)
- Mentype® AMLplus Allelic Ladder (green cap)
- DNA Size Standard 550 (BTO) (orange cap)

Prepare the denaturation mix described in Table 6 and add one or two reactions to compensate for pipetting variations. Include an extra reaction for the allelic ladder.

Table 6. Denaturation mix setup

Component	Volume per reaction
Hi-Di™ Formamide	12.0 µL
DNA Size Standard 550 (BTO)	0.5 µL
PCR product or Mentype® AMLplus Allelic Ladder (AL)	1 µL

Pipette 12.0 µL of the denaturation mixture in the wells of a PCR plate (suitable for use on the Genetic Analyzer).

Add either 1.0 µL PCR product or 1.0 µL Mentype® AMLplus Allelic Ladder (AL) into the wells. Seal the PCR plate with a suitable foil, vortex and centrifuge the plate briefly.

NOTE

Use one Mentype® AMLplus Allelic Ladder **AL** per fragment length run. Otherwise, the experiment cannot be validated.

NOTE

The capillaries of the gel electrophoresis device shall never run dry. If the samples do not occupy all capillary positions, fill the additional wells of the plate with 12.0 µL Hi-Di™ Formamide according to the capillary number.

Denature the prepared PCR products on a PCR cycler for 3 minutes at 95 °C and then cool the samples to 4 °C in the cycler. Centrifuge the samples briefly before fragment length analysis.

Fragment length analysis

Before performing the first fragment length analysis, run the **Matrix Standard BT5 multi** (BIOTYPE GmbH) to perform a spectral alignment of the used fluorescent dyes for Mentype® AMLplus Mutation Analysis Kit (6-FAM™, BTG, BTY, BTO).

NOTE

Refer to the instructions for use of Matrix Standard BT5 multi for its installation. These are available at www.biotype.de/en/ifus or upon request via support@biotype.de by BIOTYPE GmbH.

After the Matrix Standard BT5 multi has been successfully run, import the provided instrument settings for 3500 Genetic Analyzer as described in Table 7.

Table 7. provided files for Genetic Analyzers
Available at www.biotype.de/en/template-files

3500 Series Genetic Analyzers	
Instrument Protocol	POP-4™, 36 cm capillary array: AML_Instrument436.xml POP-7™, 50 cm capillary array: AML_Instrument750.xml
Size Standard Protocol	BTO_60-550_SizeStandard.xml
Sizecalling Protocol	BTO_60-550_Sizecalling.xml
Assay	POP-4™, 36 cm capillary array: AML_Assay436.xml POP-7™, 50 cm capillary array: AML_Assay750.xml

The specifications for the required instrument protocol are described in Table 8. Only described parameters should be adjusted, the other parameters should remain in the default setting. Follow the manufacturer's instructions for use to set the specific running parameters

Table 8. Parameters for the run modules of different capillary gel electrophoresis devices

	Injections Voltage [kV]	Injection Time [s]	Run Voltage [kV]	Run Time [s]
3500 Series Genetic Analyzer	3.0	8	36 cm Capillary Array: **15** 50 cm Capillary Array: **19.5**	1560
SeqStudio™ Genetic Analyzer	1.2	10	9	1560

The run time can be adjusted from the values given in Table 8, as long as all fragments (60–550 bp) of the DNA Size Standard 550 (BTO) are still analyzed.

To set up a Size Standard protocol the following sizes for DNA Size Standard 550 (BTO) must be assigned to the orange panel:

60, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp

NOTE

BIOTYPE GmbH provides specific templates for the easy installation of specific run settings for the fragment length analysis. These templates are available for download via: www.biotype.de/en/template-files.

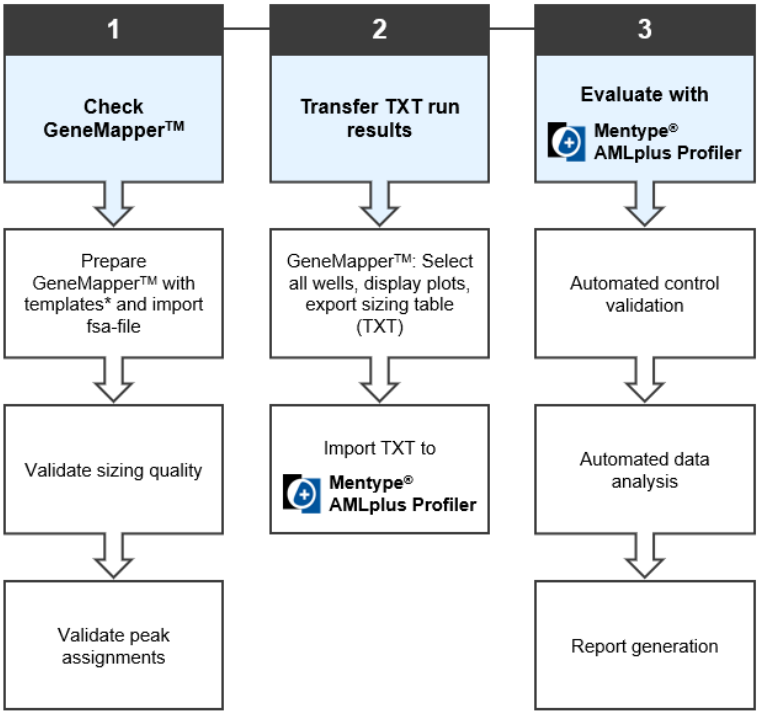
NOTE

When setting up the plate for fragment length analysis in the Genetic Analyzer data collection software, ensure that the sample names of the run controls contain the appropriate abbreviations:

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.

Data Analysis

General procedure for data analysis



Short protocol data analysis

After completion of the capillary gel electrophoresis, 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 triangle – closely review comment section of detail view
- Results of each sample are summarized within Run overview or sample-specific in detail view with further information

3.3 Report generation

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

Requirements for data analysis

The following software versions are verified for data analysis:

Pre-analysis:

- GeneMapper™ ID-X, version 1.6, Thermo Fisher Scientific
- GeneMapper® Software, version 6.1, Thermo Fisher Scientific

Data evaluation:

- Mentype® AMLplus Profiler, version 1.0.1 and higher, BIOTYPE GmbH (provided with the Mentype® AMLplus Mutation Analysis Kit)

For general instructions on these applications and sample analysis procedures, please refer to the software's user manuals.

Prepare the GeneMapper™ with the templates for data analysis as described in Table 9. All BIOTYPE templates are available on our homepage www.biotype.de/en/template-files as download or on request via support@biotype.de.

Table 9. BIOTYPE templates for GeneMapper™ Software, templates specific for [1]POP-1™, [2]POP-4™ and [3]POP-7™ polymer. Version number "v1" can be higher for updates.
*Template file is optional.

Template	GeneMapper™ 6.1	GeneMapper™ ID-X 1.6
Panels	AMLplus_Panel1_v1[1] AMLplus_Panel4_v1[2] AMLplus_Panel7_v1[3]	AMLplus_Panel1_v1x[1] AMLplus_Panel4_v1x[2] AMLplus_Panel7_v1x[3]
Bin Sets	AMLplus_Bins1_v1[1] AMLplus_Bins4_v1[2] AMLplus_Bins7_v1[3]	AMLplus_Bins1_v1x[1] AMLplus_Bins4_v1x[2] AMLplus_Bins7_v1x[3]
Stutter	-	AMLplus_Stutter1_v1x[1] AMLplus_Stutter4_v1x[2] AMLplus_Stutter7_v1x[3]
Size Standard	BTO_60-550_v1	BTO_60-550_v1x
Analysis Method	AMLplus_Analysis1_v1[1] AMLplus_Analysis4_v1[2] AMLplus_Analysis7_v1[3]	AMLplus_Analysis1_v1x[1] AMLplus_Analysis4_v1x[2] AMLplus_Analysis7_v1x[3]
Plot Setting	AMLplus_Plot1_v1[1] AMLplus_Plot4_v1[2] AMLplus_Plot7_v1[3]	AMLplus_Plot1_v1x[1] AMLplus_Plot4_v1x[2] AMLplus_Plot7_v1x[3]
Table Setting*	AMLplus_Table_v1	AMLplus_Table_v1x

NOTE

Successful allele calling and subsequent data evaluation is only guaranteed if the template files for GeneMapper™ (see Table 9) are used.

NOTE

When working with Genetic Analyzers you may experience different run behavior of fragments depending on your specific device performance and environmental factors.

You may have to adjust Panels and Bins with one or more runs of the allelic ladder on your specific instrument setup to correct deviations or missed allele calling. Contact us for support: support@biotype.de

General information on data analysis

Following the general procedure for data analysis, run and sample validation as well as data evaluation is automatically carried out by the **Mentype® AMLplus Profiler**. However, this section summarized information on the underlying assay specifications and algorithm.

DNA Size Standard 550 (BTO)

Finding the exact lengths of amplified products depends on the device type, the conditions of electrophoresis, as well as the DNA size standard used. Due to the complexity of some targets, size determination should be based on evenly distributed references.

The DNA Size Standard 550 (BTO) shall comply with the following criteria for all sample types (Sample, PC, NTC and AL):

- Presence of all fragments at: **60**, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp (see Figure 1)
- All fragments are present with **≥ 50 RFU**
- Coefficient of determination **R^2 > 0.995**
  (see Size Match Editor of GeneMapper™)
- The fragments do not continuously decrease in peak height with increasing fragment length (CE failure).

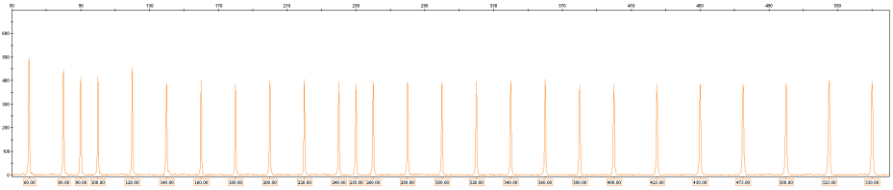


Figure 1. Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

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 10 and Figure 2). Accurate assignment requires a passing CGQ (composite genotyping quality) assessment.

Table 10. 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

Panel	Allelic ladder peak	Length [bp]		
		POP-1™	POP-4 ™	POP-7 ™
	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
	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 11 and Figure 3). 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 11. 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 (see Figure 4).

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 12. Internal Controls (IC)

Type of IC	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 (see e.g. Mentype® AMLplus Positive Control Figure 3), 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. (see e.g. Mentype® AMLplus Positive Control Figure 3)
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 (see e.g. Mentype® AMLplus Positive Control Figure 3)

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

(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 chapter Troubleshooting 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.

Troubleshooting

For accurate and reliable target detection, post-PCR analysis, including automatic target assignment and result evaluation, should be performed using the GeneMapper™ software in combination with BIOTYPE's template files and the Mentype® AMLplus Profiler. In case of any uncertainties, please contact support@biotype.de.

Peak assignments

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. Off-ladder peaks are independently detected by the Mentype® AMLplus Profiler, but artefacts labeled as target peaks may cause false-positive results.
For FLT3 ITD, fragments longer than 237 bp may still be amplified and detected but fall outside the size standard's range and must be validated individually. Visual inspection of the EPG beyond this range is recommended.

Pull-up peaks

Pull-up peaks may occur if peak heights are outside the linear detection range, or if an incorrect matrix was applied. They appear at positions of specific peaks in other color channels, typically with lower signal intensities. 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, 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 and running behavior.

Artefacts

Room temperature may influence the performance of PCR products on multi-capillary instruments; and shoulder peaks or split peaks occur. Furthermore, automated assignment could be influenced in some cases. Always consider using fresh consumables, including Hi-Di Formamide, according to the manufacturer's recommendations. Split peaks can also occur when Taq polymerase inefficiently adds the +A extension to certain amplicons. If double peaks are observed frequently (e.g., in IDH1, IDH2, CEBPA, FLT3-ITD), review the PCR protocol.
Polymer type can affect the run behavior of fragments and free fluorescent dyes, resulting in elevated background noise. These artifacts can be distinguished from true amplicons by their broad, atypical peak shape and lower signal intensity.

Competing probes are used for the **FLT3 TKD** target. Therefore, the FLT3 TKD (a) peak may sporadically show background signal across all samples, including the NTC. This does not affect target evaluation or validation if all controls were carried out in the same experiment.

When using **POP-4™** polymer, a free fluorescent dye signal has been observed within the IDH1 R132 marker region. If a false-positive call (e.g., IDH1 R132C) is suspected, confirm by CE or PCR repetition or an independent method. Additionally, Off-Ladder (OL/?) or Off-Marker-Range (OMR/?) peaks have sporadically been observed in different channels when using POP-4™. They tend to appear upstream of signal-intense amplification peaks such as NPM1, CEBPA bZIP, FLT3 wildtype, or their variants, and can be distinguished from true amplification peaks by their asymmetric, broad peak shape (Figure 2). Since OL/OMR/? peaks do not interfere with target detection, a positive result remains valid. However, if the artifact peak height exceeds 1000 RFU, CE or PCR should be repeated to ensure maximum sensitivity and accurate AR assessment of FLT3 ITD.

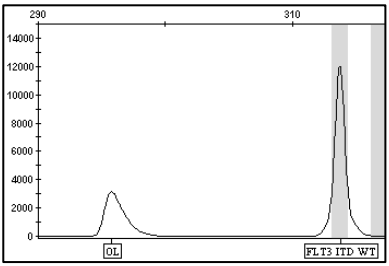


Figure 2: POP-4™ polymer. Example of one OL artefact occurring together with the FLT3 ITD WT amplicon in GeneMapper™ plots. Please note the asymmetric shape of the OL artefact in comparison to the specific amplification of FLT3 ITD WT.

Deviations in experimental setup

The robustness analysis of the Mentype® AMLplus Mutation Analysis Kit showed that the kit is robust against minor deviations from the described protocol (+/- 10 % pipetting deviation, up to 1h delay in master mix setup). Findings during the robustness analysis are as follows:

- CEBPA bZIP WT signal is sensitive to reduced polymerase concentration and fluctuations of the PCR Buffer 12 volume
- Increase of annealing temperature by 2 °C causes broad, systematic signal suppression across all targets
- Decrease of annealing temperature and different ramping increases unspecific amplifications, particularly for IDH1/2 targets.

Other abnormalities might be recognized by the software Mentype® AMLplus Profiler and lead to the following warning messages or comments as described in Table 13.

Table 13. Warning messages or comments displayed by 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.
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.

Warning message/ comment	Troubleshooting
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.
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.

Warning message/ comment	Troubleshooting
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.
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

Warning message/ comment	Troubleshooting
	due to sporadic, non-specific co-amplification, considered as background noise, and signal intense real-positive IDH1 R132G or IDH2 R172K detection
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.

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

NOTE

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

Performance Evaluation

Analytical sensitivity

Analytical sensitivity is characterized by the Limit of Detection (LoD), Limit of Blank (LoB), and Limit of Quantitation (LoQ). The LoD represents the lowest concentration of an analyte that can be reliably detected. The LoB denotes the highest result expected when analyzing a sample devoid of the analyte. The LoQ represents the lowest concentration of an analyte that can be reliably detected and quantified with acceptable precision and accuracy.

The LoD was determined under standard conditions using artificial variant mixes spiked in DNA background of human peripheral blood samples. The optimal DNA input was defined as 20 ng gDNA for all experiments, quantified by fluorometric methods for highest accuracy. The artificial targets within the mixes were tested in different concentrations around the estimated LoD in three days and three different kit lots with N= 6-8.

The LoD ranges from ≤ 1% (CEBPA bZIP INDEL) to 5% mutational content (FLT3 TKD, NPM1 TypeA/TypeB, IDH2 R140Q/R172K), depending on target (see Table 14). Not all mutation variants on a nucleic acid level detectable by the kit were individually tested in terms of sensitivity. For untested variants, an LoD of 5% mutational content, the highest result observed among tested targets, is assumed to be applicable.

Table 14. Target-specific LoDs

Target	LoD mutational content
CEBPA bZIP +3bp	1%
IDH1 R132H	2.5%
NPM1 TypeA	5%
IDH2 R140Q	5%
FLT3 ITD +3bp	2.5%
FLT3 TKD D835Y	5%
CEBPA bZIP +51bp	1%
IDH1 R132C	2.5%
NPM1 TypeB	5%
IDH2 R172K	5%
FLT3 ITD +27bp	2.5%
FLT3 TKD D835H	5%
CEBPA bZIP -18bp	0.4%
IDH1 R132G	4.1%
NPM1 TypeD	1.9%
IDH2 R140W	4.1%
IDH2 R172W	0.9%
FLT3 ITD +57bp	1.9%
FLT3 TKD I836del	5%
IDH1 R132S	4.1%
IDH1 R132L	4.1%
NPM1 TypeC	1.9%
IDH2 R140L	1.9%
FLT3 ITD +84bp	0.9%
FLT3 TKD D835V	5%

The LoB was tested on 22 samples with different specimen origin at the previously defined optimal input amount. Among them peripheral whole blood, bone marrow and periphral mononuclear blood cells (PBMCs), tested on three days in three different kit lots. The Mentype® AMLplus Mutation Analysis Kit showed no unspecific results in all the marker ranges of FLT3 TKD, CEBPA bZIP, IDH1 R132, IDH2 R172, NPM1, IDH2 R140 and

Amplification Control & FLT3 ITD with peak heights above 400 RFU. Two reoccurring off-ladder peaks were observed: a dye artefact in the green channel and a capillary electrophoresis artefact in the yellow channel, both occurring primarily with POP-4™ polymer. Both are known non-specific artefacts that do not affect the interpretation of results as they fall outside the target ranges.

The LoB experiment for the Mentype® AMLplus Mutation Analysis Kit showed no ΔFLT3 TKD ratio exceeding 0.03. Peak area shows lower result variability among negative samples compared to peak height and supports improved sensitivity for low-analyte samples. It was therefore selected for calculation of the ΔFLT3 TKD ratio and implemented in the Mentype® AMLplus Profiler. A cut-off of 0.04 was ultimately chosen to provide a conservative safety margin, accounting for the slightly higher ΔFLT3 TKD ratios observed for some wildtype samples during further internal and external site testing, still enabling sensitive variant detections as low as 5 %.

As the Mentype® AMLplus Mutation Analysis Kit is a qualitative assay designed to determine the presence or absence of the target analyte, testing the LoQ is not considered relevant.

Analytical specificity

All primers and probes in the final multiplex composition were evaluated in silico for potential cross-reactivity and non-specific amplification against the human genome GRCh38, as well as for primer dimer and hairpin formation. No off-target amplification or interactions were identified that would be expected to affect assay performance.

Known single nucleotide polymorphisms (SNPs) located within primer binding sites were also evaluated for the risk of false-negative results due to primer mismatches. The majority of identified SNPs occur at very low population frequency (<0.001) or are located away from the primer's 3' end, where mismatches have the greatest impact on amplification. One SNP (rs377040701) was identified directly at a primer's 3' end for FLT3 TKD target evaluation, occurring at 0,02% frequency within the African population; given this low frequency combined with the low frequency of TKD mutations (~7%), the risk to decreased assay performance is <0.001 and therefore not considered relevant.

Assay Cut-off

Based on the results of the LoB study, a peak-height threshold of ≥ 400 RFU was established as the criterion for valid target detection. It was confirmed as robust with four tested devices.

For detection of FLT3 TKD mutations, a ΔFLT3 TKD ratio of > 0.04 must additionally be achieved.

In-use stability

In-use freeze-thaw stability of the Mentype® AMLplus Mutation Analysis Kit was evaluated across multiple freeze-thaw cycles using artificial sample mixtures in a wildtype peripheral blood background, covering the assay's relevant target markers. All peak heights and semi-quantitative ratio parameters (FLT3 ITD area ratio, ΔTKD ratio) remained within the defined acceptance criteria throughout testing, with no systematic degradation trend observed across the tested cycle range. Based on these results, the kit demonstrates robust performance under repeated freeze-thaw handling as encountered in routine use for up to 20 freeze-thaw cycles.

Quality Control

All kit components undergo an intensive quality assurance process at BIOTYPE GmbH. Quality of the test kits is permanently monitored to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance

Technical Assistance

For technical advice, please contact our Customer Support Team:

e-mail: support@biotype.de

phone: +49 (0)351 8838 400

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

Limitations of Use

- The procedures in this handbook must be followed, as described. Any deviations may result in assay failure or cause erroneous results.
- Use of this product is limited to laboratory professional users specially trained in PCR techniques and capillary gel electrophoresis.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The kit has only been verified for use with the reagents, instruments and software described in chapter Material and devices required but not provided
- Good laboratory practice is required to ensure the performance of the kit.
- Results must be interpreted by a trained healthcare professional.
- Interpretation of results must account for the possibility of false negative and false positive results.
- Do not use expired or incorrectly stored components

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

Trademarks and Disclaimers

Mentype® is a registered trademark of BIOTYPE GmbH.

Other trademarks: GeneMapper™, SeqStudio™ and Applied Biosystems® (Applied Biosystems LLC group); Qubit® (Thermo Fisher Scientific), QIAamp® (Qiagen); POP-4™, POP-1™, POP-7™ (Europe: Applied Biosystems LLC, US: Life Technologies Corporation), NucleoSpin® (Macherey-Nagel). 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.

Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

Not available in all countries.

Explanation of Symbols

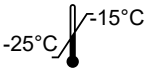
Manufacturer

Batch code

Contains sufficient reagents for <N> tests

Reference to eIFU

Use by



Storage temperature limitation

Catalogue number

RUO

For research use only. Not for use in diagnostic procedures.

Protect from light

Keep dry

Further marking used in this Instruction for Use:

	
	Attention, be sure to follow this notice!
blue underlined text	Links leading to external content like homepages, e-mail addresses
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

Electropherogram of Mentype® AMLplus Allelic Ladder

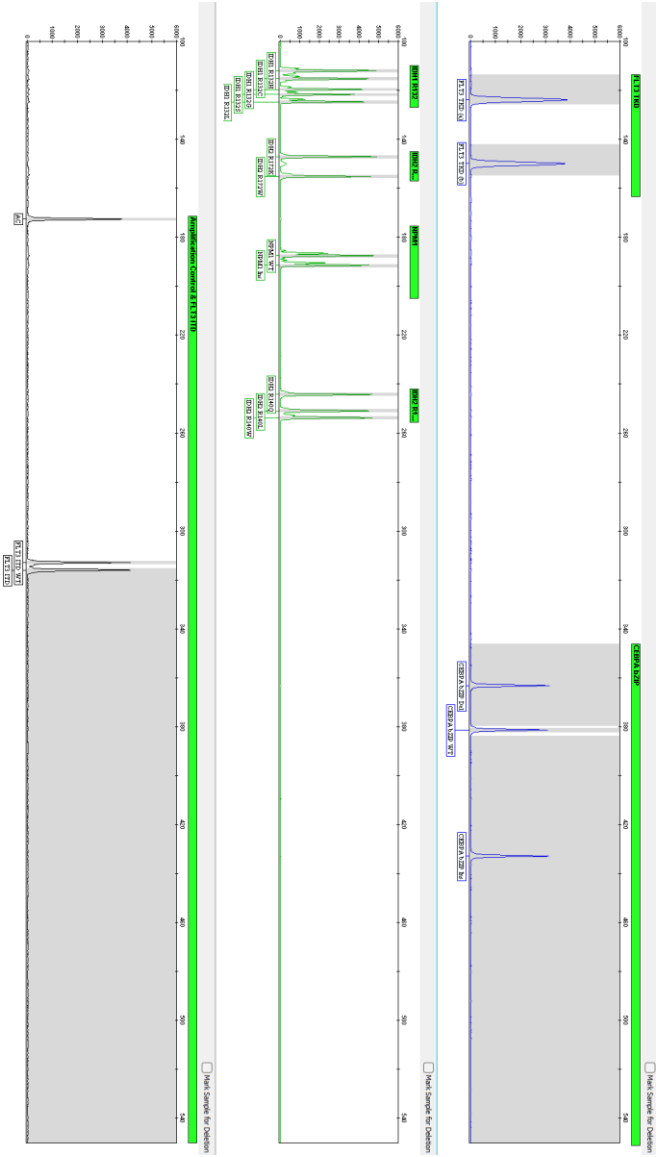


Figure 2. Mentype® AMLplus Allelic Ladder.
Analysis on POP-7™ polymer. Blue, green and yellow channel.

Electropherogram of Mentype® AMLplus Positive Control

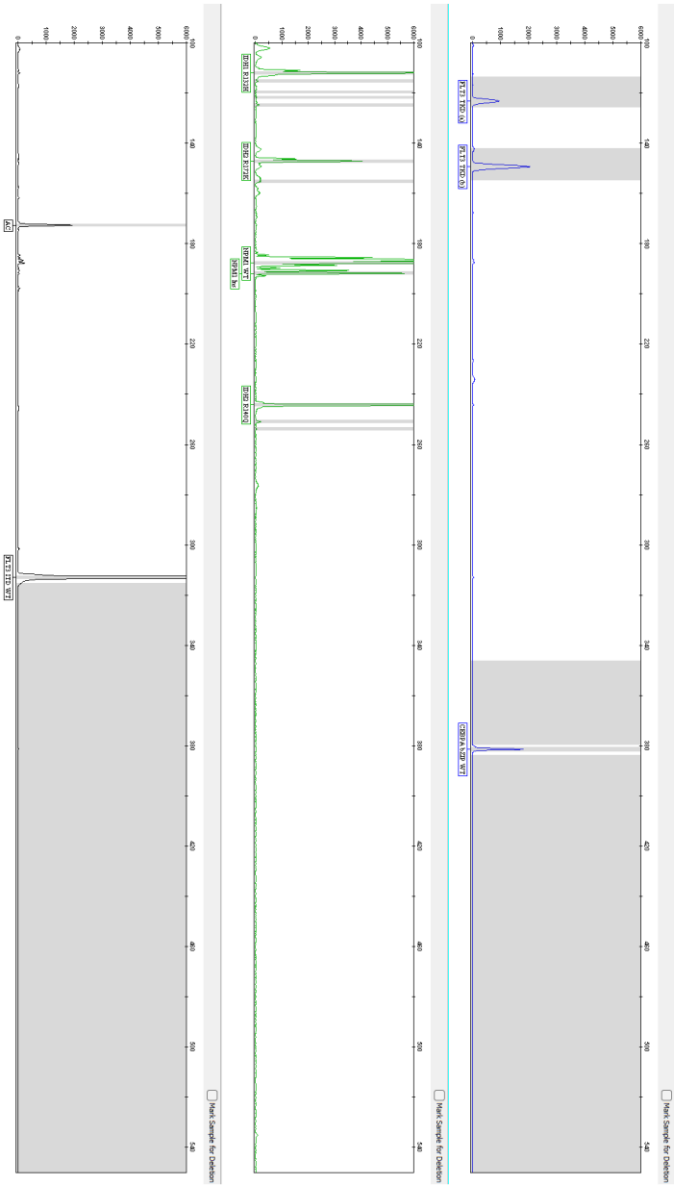


Figure 3. Mentype® AMLplus Positive Control.
Analysis on POP-7™ polymer. Blue, green and yellow channel.

Electropherogram of No-Template Control

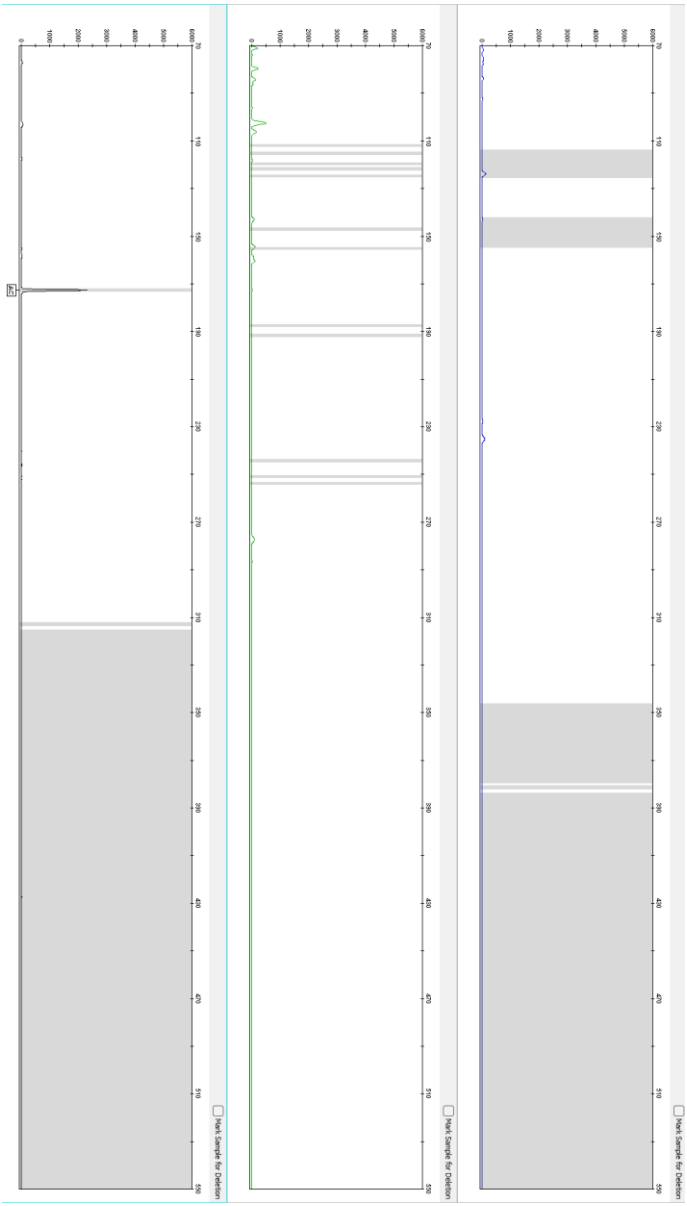


Figure 4. No-Template Control (NTC)
Analysis on POP-7™ polymer. Blue, green and yellow channel.

Table 15. Target gene list
Genome Reference GRCh38

Gene symbol (HGNC)	Alias and previous symbols	Ensembl Gene ID	Targeted Region (5’ to 3’)
NPM1	B23; NPM	ENSG00000181163	NM_002520.7:c.847-54_*91 (Exon11 + 3'UTR)
FLT3	CD135; FLK2; STK1	ENSG00000122025	ITD: NM_004119.3:c.1705-12_1912 (includes Intron) TKD: NM_004119.3:c.2493_2512
CEBPA	C/EBPα; CEBP	ENSG00000245848	NM_004364.5:c.816_*123/ bZIP-Domain p.293-356 + 3'UTR
IDH1	IDH; IDPC	ENSG00000138413	NM_005896.4: c.394_396
IDH2	IDH-2; IDPM; IDH	ENSG00000182054	R140: NM_002168.4:c.418_420 R172: NM_002168.4:c.514_516

BIOTYPE GmbH

Moritzburger Weg 67
01109 DRESDEN
GERMANY
Tel.: +49 351 8838 400
Fax: +49 351 8838 403
www.biotype.de

Ordering
sales@biotype.de

Customer Service & Support
support@biotype.de