Mentype® AMLplex^{QS}

PCR Amplification Kit

Instructions for Use (IFU)

IVD CE 0483 For in vitro diagnostic use

AMLIFU02v2en
23.07.2025

REF 45-12100-0025
45-12100-0100
45-12100-0400

LOT Batch code

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 IFU version:

Document code	Changes	Date
AMLIFU02v1en	Initial version	28.05.2025
AMLIFU02v2en	Update table 8	23.07.2025

A printed version of this IFU can be provided free of charge within 7 days.

For any further questions, please contact us

at +49 351 8838 400 or support@biotype.de

Content

Intended purpose

The Mentype® AMLplex^{QS} PCR Amplification Kit is a manual assay intended to be used for the qualitative screening of 11 gene fusions with a total of 34 transcript variants, enabling the identification of chromosomal aberrations with diagnostic, prognostic and therapeutic relevance for adult patients suffering or who are suspected of suffering from Acute Myeloid Leukemia (AML).

The assay is to be used with complementary DNA (cDNA), which has been reversely transcribed from cellular RNA that was extracted from peripheral venous whole blood or bone marrow samples.

The Mentype® AMLplex^{QS} PCR Amplification Kit is used to detect chromosomal aberrations for clinical decisions relevant in Acute Myeloid Leukemia. The identification of the specific genetic translocations enables the classification of leukemic diseases and provides essential information for the diagnostic rationale determining risk-directed therapy for patients.

The Mentype® AMLplex^{QS} PCR Amplification Kit is intended for professional laboratory users trained on molecular-genetic techniques, multiplex PCR and the handling of Genetic Analyzer of Thermo Fisher Scientific (Applied Biosystems division).

Scientific background

The verification of specific chromosomal aberrations has high prognostic value in nearly all types of acute leukemia. Molecular biological evidence of chromosomal aberrations (translocations) represents an important diagnostic completion. Detecting specific translocations enables the subtype classification of leukemic diseases and provides essential information for the risk-directed therapy of patients.

The Mentype® AMLplex^{QS} PCR Amplification Kit facilitates the detection of the most common chromosomal aberrations observed so far in AML and represents a simple-to-use, routine-compatible and reliable screening tool.

Product description

The Mentype® AMLplex^QS PCR Amplification Kit contains optimized reagents for the high-resolution detection of 11 fusion genes with 34 transcript variants in total (see Table 1). The naming of variants follows historically established nomenclatures, especially for KMT2A (Schnittger et al., 2000). A complete list of genes, their alternative names and the transcript IDs used for systematic variant descriptions can be found in the Appendix (See Target gene list).

The test kit includes an internal PCR control (Quality Sensor "QS-Control"), that is template independent and a template dependent cDNA Control (ABL-Control), providing helpful information about PCR efficiency, the quality of applied cDNA templates, and the presence of PCR inhibitors.

The test is performed by fragment analysis using capillary gel electrophoresis. One primer for each transcript is fluorescence-labelled with 6-FAM, BTG or BTY, the color spectra of the accessory Matrix Standard BT5 multi (BIOTYPE, GmbH).

Table 1 Gene fusions and transcript variants detectable with Mentype® AMLplex^QS PCR Amplification Kit

Gene fusion	Chromosomal aberration	Variant	Systematic variant description
RUNX1::RUNX1T1	t(8;21) (q22;q22.1)	-	RUNX1:e6::RUNX1T1:e3
BCR::ABL	t(9;22) (q34.1;q11.2)	e1a3 e1a2 b3a2 b3a3 b2a2 b2a3	BCR:e1::ABL1:e3 BCR:e1::ABL1:e2 BCR:e14::ABL1:e2 BCR:e14::ABL1:e3 BCR:e13::ABL1:e2 BCR:e13::ABL1:e3
PICALM::MLLT10	t(10;11) (p12.3;q14.2)	MLLT10_240-PICALM_1987 MLLT10_240-PICALM_2092	MLLT10:e3::PICALM:e19 MLLT10:e3::PICALM:e20
CBFB::MYH11	inv(16) (p13.1;q22)	Type A Type B Type C Type D Type E Type F Type G Type H Type I Type J	CBFB:e5::MYH11:e33 CBFB:e5::MYH11:e32 CBFB:e5::MYH11:e31 CBFB:e5::MYH11:e29 CBFB:e5::MYH11:e28 CBFB:e4::MYH11:e33 CBFB:e4::MYH11:e29 CBFB:e4::MYH11:e28 CBFB:e4::MYH11:e34 CBFB:e5::MYH11:e30

Gene fusion	Chromosomal aberration	Variant	Systematic variant description
DEK::NUP214	t(6;9) (p23.3;q34.1)	-	DEK:e9::NUP214:e18
KMT2A::MLLT4	t(6;11) (q27;q23.3)	-	KMT2A:e8::AFDN:e3
KMT2A::MLLT3	t(9;11) (p21.3;q23.3)	6A (6A_S; 6A_L)	KMT2A:e8::MLLT3:e6
		7A	KMT2A:e8::MLLT3:e7
		8A	KMT2A:e8::MLLT3:e8
		6B	KMT2A:e8::MLLT3:e6
KMT2A::ELL	t(11;19) (q23.3;p13.1)	e10e2	KMT2A:e8::ELL:e2
		e10e3	KMT2A:e8::ELL:e3
KMT2A-PTD	Partial Tandem Duplication	e9e3	KMT2A:e8::KMT2A:e2
		e10e3	KMT2A:e9::KMT2A:e2
		e11e3	KMT2A:e10::KMT2A:e2
NPM1::MLF1	t(3;5) (q25.3;q35.1)	-	NPM1:e6::MLF1:e2
PML::RARA	t(15;17) (q24.1;q21.2)	bcr1	PML:e6::RARA:e3
		bcr2	PML:e5::RARA:e3
		bcr3	PML:e3::RARA:e3

The assay was validated by gene fusion screening of ~ 300 AML patients and its suitability was confirmed in a comparative clinical evaluation study.

The detection limit for the qualitative assessment is 400 RFU. On average a minimum of 1000 copies can be detected for each gene fusion.

The general input range under standard conditions into the reverse transcription reaction is defined as 100 ng to 1 µg total RNA isolated from whole blood or bone marrow.

The optimal input into reverse transcription for bone marrow is defined as 500 ng RNA and 1 µg RNA for peripheral blood. 1 µL of cDNA is then used for PCR.

Materials provided

Table 2 Mentype® AMLplex^QS PCR Amplification Kit content

Reagent	Cap color	Volume per packaging size		
		25 reactions	100 reactions	400 reactions
Nuclease-Free Water	Light blue	1.5 mL	2 x 1.5 mL	6 x 1.5 mL
Reaction Mix A	Purple	125 µL	500 µL	2 x 1.0 mL
Mentype® AMLplex^QS Primer Mix	Red	63 µL	250 µL	4 x 250 µL
Multi Taq 2 DNA Polymerase	White	10 µL	40 µL	160 µL
Mentype® AMLplex^QS Positive Control	White	25 µL	25 µL	25 µL
DNA Size Standard 550 (BTO)	Orange	13 µL	50 µL	200 µL
Mentype® AMLplex^QS Allelic Ladder	Green	25 µL	25 µL	4 x 25 µL

An overview of the component batch numbers can be found on the label which is situated on the inside of the box flap.

NOTE

Please note that the packaging size describes the number of tests **without** taking into account the number of required controls or the required excess for pipetting.

We recommend to use the following size for the corresponding throughput:

- < 8 samples per PCR run: 25 reaction packaging size
- 8 - 45 samples per PCR run: 100 reaction packaging size
- > 45 samples per PCR run: 400 reaction packaging size

Description of components

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

Reaction Mix A: PCR buffer containing dNTPs and $MgCl_2$. The PCR buffer is optimized to promote enzyme activity for the PCR.

Mentype® AMLplex^{QS} Primer Mix: multiplex oligonucleotide primer mix containing labeled primer (label: 6-FAM™, BTG, BTY) and unlabeled primers.

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

Mentype® AMLplex^{QS} Positive Control: double stranded artificial DNA mixture of five DNA-fragments. It is used as a qualitative, external PCR control, which is positive for the following targets on all 3 panels: BCR::ABL_b2a3, RUNX1::RUNX1T1, ABL-Control, KMT2A-PTD_e11e3 and PML::RARA_bcr3, additionally the QS-Control is amplified as a quality marker for sample validity.

NOTE

The Mentype® AMLplex^{QS} 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, it is used for a size regression to exactly determine the fragment length of the PCR products.

Mentype® AMLplex^{QS} Allelic Ladder: a mixture of fluorophore-labeled PCR fragments representing all of the detectable alleles of Mentype® AMLplex^{QS} PCR Amplification Kit. It includes 22 fragments labeled with 6-FAM™, 12 fragments labeled with BTG and 2 fragments labeled with BTY, used as a genotyping reference for the exact allele identification.

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.

Please check the completeness of the kit upon receipt. Do not use kits that have been thawed upon arrival. If one or more components 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® AMLplex^{QS} Primer Mix, DNA Size Standard 550 (BTO) and Mentype® AMLplex^{QS} Allelic Ladder must be stored protected from light.

In order to prevent contamination, we recommend that pre-amplification components (RNA and cDNA samples, the Mentype® AMLplex^{QS} Positive Control) and the post-amplification components (DNA Size Standard 550 (BTO) and Mentype® AMLplex^{QS} Allelic Ladder) are stored and used separately from PCR reagents (Nuclease-Free Water, Multi Taq 2 DNA Polymerase, Reaction Mix A and Mentype® AMLplex^{QS} Primer Mix).

The kit will expire according to the information on the kit box label or 24 months after opening, whichever comes first. 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

- NanoDrop™ One Spectrophotometer or 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
RNeasy Mini Kit	Qiagen GmbH	74104
RNeasy Plus Mini Kit	Qiagen GmbH	74134
RNeasy Midi Kit	Qiagen GmbH	75144
NucleoSpin Dx RNA Blood (50 preparations)	Macherey-Nagel	740201.50
Maxwell® CSC RNA Blood Kit (48 preparations)	Promega	AS1410
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor	Thermo Fisher Scientific	4374966 4374967
SuperScript™ IV First Strand cDNA Synthesis System	Thermo Fisher Scientific	18091050, 18091200
Hi-Di™ Formamide, 25 mL	Thermo Fisher Scientific	4311320
POP-4™ Polymer for 3500/3500xL Genetic Analyzers (384 samples)	Thermo Fisher Scientific	4393715
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

Reagent	Supplier	Order number
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 manual column-based extraction methods as described in chapter Sample preparation, the following automation can be used for RNA extraction:

- Maxwell® CSC device (cat. no.: AS6000, Promega)

The Mentype® AMLplex^QS PCR Amplification Kit was validated 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 Silver (discontinued, Thermo Fisher Scientific)
- Mastercycler nexus gradient (cat. no.: 6331000017, Eppendorf AG)
- Mastercycler ep-S (discontinued, Eppendorf AG)
- Biometra Tadvanced (cat. no.: 846-2-070-211; Analytik Jena)

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

- Heated lid
- Block suitable for 200 µL reaction plates / tubes
- Ramping adjustable to 4 °C/s

The Mentype® AMLplex^QS PCR Amplification Kit was validated 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:

- GeneMapper™ ID-X software, version 1.6 (Thermo Fisher Scientifc), using one of the product specific (see also Table 11):
  - Analysis Methods: AMLplexIVD_Analysis1_v1x or AMLplexIVD_Analysis4_v1x or AMLplexIVD_Analysis7_v1x
  - Bins: AMLplexIVD_Bins1_v1x or AMLplexIVD_Bins4_v1x or AMLplexIVD_Bins7_v1x
  - Panels: AMLplexIVD_Panel1_v1x or AMLplexIVD_Panel4_v1x or AMLplexIVD_Panel7_v1x
  - Size Standard: BTO_60-550_v1x

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.

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

Specimens and test samples

The following human specimen have been validated with the Mentype® AMLplex^{QS} PCR Amplification Kit :

- Peripheral venous blood samples (EDTA, citrate, heparin and stabilizer RNA Exact), stored at 4 °C and processed for RNA purification within 24 h.
- Bone marrow samples (EDTA, heparin), stored at 4 °C and processed for RNA purification within 24 h.

The obtained RNA shall be stored undiluted at -85 °C to -70 °C.

NOTE

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

Warnings and precautions

- Read the Instructions for Use 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.
- The use of this product is limited to laboratory professional users, trained on molecular-genetic techniques, multiplex PCR, and the handling of Genetic Analyzers of Thermo Fisher Scientific.
- 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.

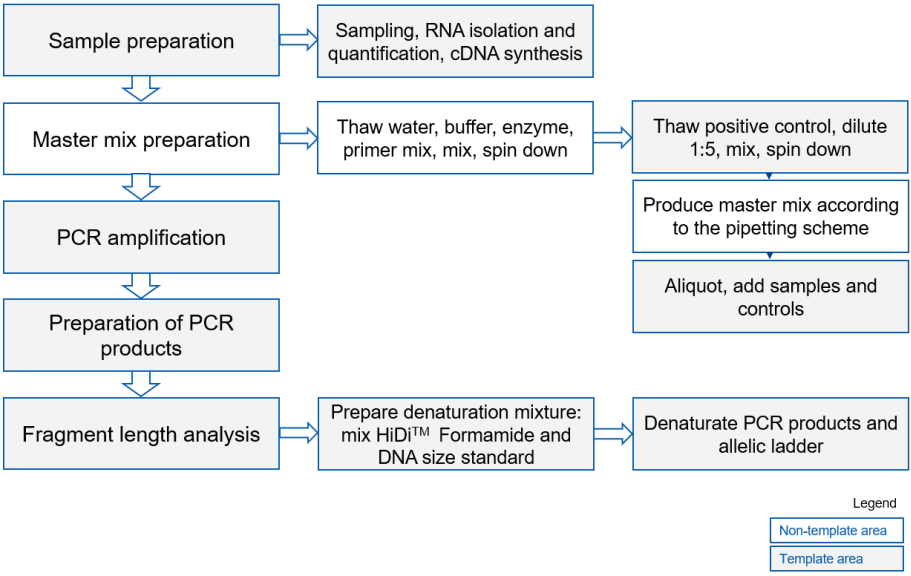
- All instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

Notice to the user

Any problem that occurs in relation to the product shall be reported by the user to the manufacturer. Any serious incidents related to this kit must be reported to the manufacturer and the appropriate authority of the member states in which the user and/or the patient is established. A Summary of Safety and Performance (SSP) is created in accordance with Article 29 of Regulation (EU) 2017/746 and intended to provide public access via EUDAMED database to an updated summary of data on safety and performance of the device to intended users, in the case of this product laboratory professionals only.

Procedure

Overview of the experimental workflow



Sample preparation

Raw sample requirements

The specimen for the Mentype® AMLplex^QS PCR Amplification Kit is defined as cDNA transcribed from RNA extracted from peripheral venous whole blood or bone marrow collected from humans.

We recommend the use of reagents for RNA stabilization during sample collection. RNA stabilizers help to preserve RNA integrity (e. g. S-Monovette RNA Exact). RNA isolation should be performed promptly after sample collection.

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

Take at least a 200 µL sample of peripheral venous whole blood for the following procedure.

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

Bone marrow

Take at least a 200 µL sample of bone marrow for the following procedure.

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 stored

and transported at 4 °C. RNA should be extracted within 1 h - 4 h after sampling for best results.

RNA extraction

Perform the RNA extraction and purification from peripheral venous whole blood (PB) or bone marrow (BM) aspirates with one of the following kits:

- RNeasy® Midi/ Mini/ Plus Mini Kit (Qiagen GmbH) for anticoagulants EDTA, Citrate and Heparin on BM and PB
- NucleoSpin® Dx RNA Blood (Macherey-Nagel) for stabilizer RNA Exact on PB
- Maxwell® CSC RNA Blood Kit (Promega) for anticoagulants EDTA and Heparin on BM

Please follow the manufacturer's manuals and recommendations for RNA extraction. It is recommended to perform a **DNase digestion** during isolation according to manufacturer's instruction.

NOTE

Blood contamination can be visually detected in the PCR reaction or in the isolated RNA by an orange color shift. If a color change is observed, we recommend repeating the RNA 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.

RNA quantification and dilution

Quantify the RNA concentration by UV/VIS spectroscopy at 260 nm using the NanoDrop™ One Spectrophotometer or by fluoroscence spectroscopy using the Qubit™ Fluorometer.

When using spectrophotometry, use the elution buffer from the RNA extraction kit to measure the blank. The A260/A280 ratio shall be in the range of 1.9 - 2.1, whereas the A260/A230 ratio shall be in the range of 1.8 – 2.3.

For the fluorometric quantification of the RNA the Qubit™ Fluorometer with either the Qubit™ RNA HS Assay-Kit or Qubit™ RNA BR Assay-Kit can be used.

For use with Mentype® AMLplex^{QS} PCR Amplification Kit, dilute the RNA samples to a suitable concentration for the subsequent transcription into cDNA. Prepare the dilution freshly before usage. Use nuclease-free water as the diluent.

NOTE

The input range for the transcription into cDNA follows the instructions from the transcription kit manufacturer. Usages of 100 ng - 1 µg RNA in a final reaction volume of 20 µL for the cDNA synthesis is validated for both sample types. We recommend using an optimum of **500 ng RNA for bone marrow** and **1 µg RNA for peripheral blood.**

RNA storage

Store the undiluted RNA samples in RNAse-free water at -25 °C to -15 °C up to 24 hours or -85 °C to -70 °C for up to one year.

Transcription into cDNA

Perform the cDNA synthesis according to the manufacturer's instructions, using one of the following kits:

- High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Thermo Fisher Scientific)
- SuperScript™ IV First Strand cDNA Synthesis System* (Thermo Fisher Scientific)

*recommended for low RNA quantity

NOTE

Heat inactivation of reverse transcriptase is crucial to PCR performance. Please refer to the manufacturer‘s instructions for detail.

cDNA storage

Store the cDNA samples at -25 °C to -15 °C for up to one year.

Control preparation

Positive control (PC)

Thaw the Mentype® AMLplex^{QS} Positive Control, homogenize it by careful vortexing followed by a brief centrifugation.

Dilute the Mentype® AMLplex^{QS} Positive Control **1:5** using the Nuclease-Free Water included in the kit. E. g. mix 1 µL of the control with 4 µL of Nuclease-Free Water.

Homogenize the diluted PC by careful vortexing. After this, briefly centrifuge the diluted PC (approx. 5 s).

Do not store the diluted positive control.

NOTE

Always apply a fresh dilution of the Mentype® AMLplex^{QS} Positive Control.

No template control (NTC)

Apply the Nuclease-Free Water included in the kit as no template control NTC instead of a sample.

Master mix setup

Remove and thaw the following components from the Mentype® AMLplex^QS PCR Amplification Kit :

- Nuclease-Free Water (light blue cap)
- Reaction Mix A (purple cap)
- Mentype® AMLplex^QS Primer Mix (red cap)
- Multi Taq 2 DNA Polymerase (white cap)

All frozen components need to be thawed at room temperature (22 °C to 25 °C, ca. 30 min, protected from light) and homogenized by inverting the tubes, pipetting, or gently vortexing. After this, briefly centrifuge the reagents (approx. 5 s). Prior to the master mix setup, it is recommended to keep the Multi Taq 2 DNA Polymerase in a cooled environment as long as possible (e. g. cooling rack).

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

Component	Volume		
	# 1	# 5	# 10
Nuclease-Free Water*	16.1 µL	80.5 µL	161.0 µL
Reaction Mix A	5.0 µL	25.0 µL	50.0 µL
Mentype® AMLplex^QS Primer Mix	2.5 µL	12.5 µL	25.0 µL
Multi Taq 2 DNA Polymerase	0.4 µL	2.0 µL	4.0 µL
cDNA 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 to 2 µL, 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.

Application of cDNA templates 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.

cDNA template: add 1.0 µL of the undiluted cDNA samples.

PC: add 1.0 µL of the freshly prepared, 1:5 diluted Mentype® AMLplex^QS Positive Control instead of a sample.

NOTE

First, prepare the NTC to avoid contaminations of the control. Prepare the PC as a last to avoid cross contaminations of the samples.

NOTE

Use at least one positive control PC and one no template control NTC per run. Otherwise, the run cannot be validated.

Close all PCR tubes, gently vortex and spin down.

PCR amplification

Program the PCR cycler with the following amplification profile (see Table 5), make sure to set the ramping to 4 °C/s. Perform a “hot start” PCR in order to activate the polymerase and to prevent the formation of non-specific amplification products.

NOTE

Using calibrated and well-maintained PCR cyclers is essential, as deviations can negatively affect the amplification of sensitive targets, such as KMT2A::MLLT4.

Table 5 PCR protocol

Temperature	Time	
96 °C	4 min (hot start for activation of the polymerase)	
96 °C	30 s	
60 °C	120 s	25 cycles
72 °C	75 s	
68 °C	10 min*	
10 °C	∞	hold

* If a higher amount of minus -Adenine peaks (-1 bp) is observed, extension up to 60 min is possible.

NOTE

If thermal cyclers with adjustable heating and cooling rates are used, **ramping shall be adjusted to 4 °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 4 weeks at 2 °C to 8 °C or long-term at -25 °C to -15 °C protected from light.

Thaw, mix and centrifuge the reagents:

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

Component	Volume per reaction
Hi-Di™ Formamide	12.0 µL
DNA Size Standard BTO (550)	0.5 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® AMLplex^{QS} Allelic Ladder into the wells. Seal the PCR plate with a suitable foil, vortex and centrifuge the plate briefly.

NOTE

The allelic ladder is used to correctly determine the fragments analyzed during data analysis. In each fragment length analysis run, the allelic ladder must be analyzed at least once to ensure successful data analysis.

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® AMLplex^{QS} PCR Amplification 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 (www.biotype.de/en/template-files).

Table 7 provided files for Genetic Analyzers (www.biotype.de/en/template-files)

3500 Series Genetic Analyzers	
Instrument Protocol	POP-4™, 36 cm capillary array: AMLplexIVD_Instrument436.xml POP-7™, 50 cm capillary array: AMLplexIVD_Instrument750.xml
Size Standard Protocol	BTO_60-550_SizeStandard3500.xml
Sizecalling Protocol	BTO_60-550_Sizecalling.xml
Assay	POP-4™, 36 cm capillary array: AMLplexIVD_Assay436.xml POP-7™, 50 cm capillary array: AMLplexIVD_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 the 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

Differing from the values given in Table 8, the run time can be adjusted: it must be ensured that all fragments (60 - 550 bp) of the DNA Size Standard 550 (BTO) are analyzed.

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

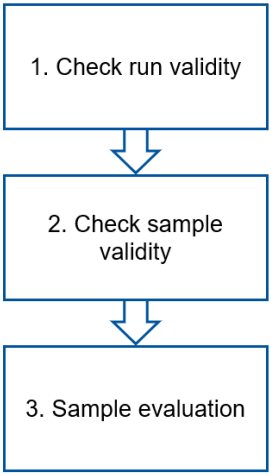
60, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

NOTE

BIOTYPE GmbH provides specific templates for the easy installation of specific run settings for the fragment length analysis as well as analysis templates for a simple software set-up of GeneMapper™ ID-X. These templates are available for download via: www.biotype.de/en/template-files.

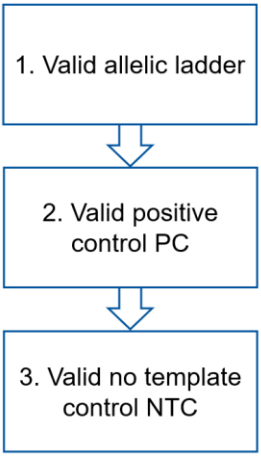
Data Analysis

General procedure for the data analysis



- See chapter **General run validation**
- See chapter **Sample analysis**
- See chapter **Sample analysis**

General run validation



- Valid DNA Size Standard 550 (BTO) ≥ 50 RFU
- Valid peak height ≥ 400 RFU
- All expected peaks present
- Valid DNA Size Standard 550 (BTO) ≥ 50 RFU
- Valid peak height ≥ 400 RFU
- All expected peaks present
- Valid DNA Size Standard 550 (BTO) ≥ 50 RFU
- No peaks ≥ 400 RFU detected

NOTE

The whole measuring range from 50 bp to 560 bp should be analyzed to assess validity.

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.

Check the DNA Size Standard 550 (BTO) in all samples for the following criteria:

- 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™ ID-X)
- The fragments do not continuously decrease in peak height with increasing fragment length.

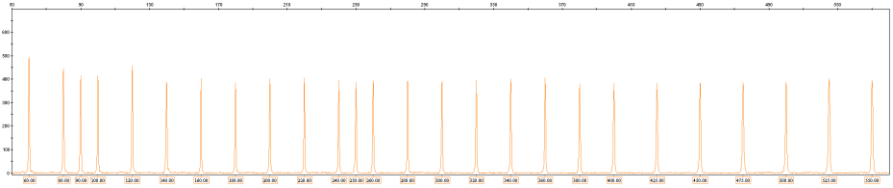


Figure 1 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Mentype® AMLplex^QS Allelic Ladder

After ensuring a valid size standard, check that all peaks in the allelic ladder are present with **≥ 400 RFU**.

NOTE

The Mentype® AMLplex^QS Allelic Ladder includes one peak for each deteactable target. Please compare the alleles with appendix Figure 2.

Mentype® AMLplex^QS Positive Control (PC)

After ensuring a valid size standard, make sure that all specific peaks for the PC are present with **≥ 400 RFU**.

The Mentype® AMLplex^QS Positive Control, which is part of the test kit, represents the following targets (see Table 9).

Table 9 Control peaks in Mentype® AMLplex^QS Positive Control

Panel	Control peak
Blue	▪ QS-Control ▪ BCR::ABL_b2a3 ▪ RUNX1::RUNX1T1 ▪ ABL-Control
Green	▪ KMT2A-PTD_e11e3
Yellow	▪ PML::RARA_bcr3

NOTE

The Mentype® AMLplex^QS Positive Control includes at least one peak for each panel used. Please compare the alleles with appendix Figure 3.

No template control (NTC)

After ensuring a valid size standard, check that no peaks **≥ 400 RFU** are detected in the NTC (see also appendix Figure 4).

NOTE

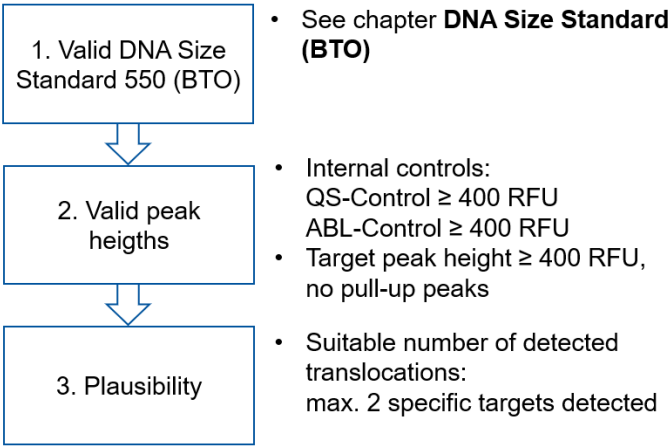
Using GeneMapper™ ID-X together with the provided template files for the Analysis Method, peaks < 400 RFU in Mentype® AMLplex^{QS} PCR Amplification Kit samples are automatically not assigned with the allele name supporting you to easily evaluate the NTC.

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 amplicon peaks is possible.

Sample analysis

Workflow data analysis



NOTE

If multiple target detections exceed the cut-off or if you encounter any uncertainties, please check the troubleshooting section (e. g. for a list of known signal combinations) or reach out to customer support at support@biotype.de.

Using GeneMapper™ ID-X software together with the specific templates provided by BIOTYPE GmbH, the basic validation is done automatically.

After checking the run and sample validity, the sample data have to be evaluated.

NOTE

The Mentype® AMLplex^QS is an **exclusively qualitative test**. This application is not suited to quantify copy numbers or monitor Minimal Residual Disease (MRD).

When using the evaluation templates of BIOTYPE GmbH and after successful evaluation of the Allelic Ladder of the run, the detected PCR fragments are named automatically. An overview of the fragment lengths of the PCR products can be found in the following Table 10.

Table 10 Overview over the fragment lengths of the translocations in the Mentype® AMLplex^QS Allelic Ladder on POP-4™ polymer. ‡ Two amplicons for variant KMT2A::MLLT3_6A; * Although this variant is detectable with Mentype® AMLplex^QS PCR Amplification Kit, the varying length of the amplicon (approx. 173 bp) prevents automated allocation

Panel/ Translocation	Length [bp]	Panel/ Translocation	Length [bp]
FAM Panel (Blue Channel)		**BTG Panel (Green Channel)**	
CBFB::MYH11_TypeG	63	DEK::NUP214	78
CBFB::MYH11_TypeI	66	KMT2A-PTD_e9e3	87
QS-Control	72	KMT2A::MLLT3_6A_S‡	113
BCR::ABL_b2a3	107	KMT2A::MLLT3_6B	190

Panel/ Translocation	Length [bp]	Panel/ Translocation	Length [bp]
CBFB::MYH11_TypeJ	141	KMT2A-PTD_e10e3	217
CBFB::MYH11_TypeC	146	KMT2A::ELL_e10e3	241
CBFB::MYH11_TypeD	160	KMT2A::MLLT3_7A	245
CBFB::MYH11_TypeH	165	KMT2A::ELL_e10e2	289
CBFB::MYH11_TypeF	175	KMT2A::MLLT4	303
BCR::ABL_b3a3	183	KMT2A-PTD_e11e3	332
BCR::ABL_e1a3	206	KMT2A::MLLT3_8A	359
MLLT10_240::PICALM_2092	265	KMT2A::MLLT3_6A_L‡	497
CBFB::MYH11_TypeA	271	**BTY Panel (Yellow Channel)**	
BCR::ABL_b2a2	282	PML::RARA_bcr1	220
RUNX1::RUNX1T1	301	PML::RARA_bcr3	290
BCR::ABL_b3a2	357	PML::RARA_bcr2*	
CBFB::MYH11_TypeE	366		
MLLT10_240::PICALM_1987	371		
BCR::ABL_e1a2	380		
NPM1::MLF1	389		
CBFB::MYH11_TypeB	485		
ABL-Control	519		

Data analysis with GeneMapper™ ID-X

Preparation of GeneMapper™ ID-X software

For general instructions on the application and sample analysis with this software, please refer to the GeneMapper™ ID-X Software user's manual.

The allele allocation shall be carried out with the analysis software GeneMapper™ ID-X in combination with the Mentype® AMLplex^{QS} PCR Amplification Kit template files from BIOTYPE GmbH. The BIOTYPE

template files (see Table 11) are available on our homepage (www.biotype.de/en/template-files) as download or on request via support@biotype.de. The analysis workflow using the GeneMapper™ ID-X software is shown in Table 12.

Table 11 BIOTYPE GmbH templates for GeneMapper™ ID-X Software, templates specific for [1]POP-1™, [2]POP-4™ and [3]POP-7™ polymer

Template	Template name	
Panels*	AMLplexIVD_Panel1_v1x[1] AMLplexIVD_Panel4_v1x[2] AMLplexIVD_Panel7_v1x[3]	or higher versions
Bin sets*	AMLplexIVD_Bins1_v1x[1] AMLplexIVD_Bins4_v1x[2] AMLplexIVD_Bins7_v1x[3]	or higher versions
Size Standard*	BTO_60-550_v1x	or higher versions
Analysis Method*	AMLplexIVD_Analysis1_v1x[1] AMLplexIVD_Analysis4_v1x[2] AMLplexIVD_Analysis7_v1x[3]	or higher versions
Plot Settings	PlotsBT5_4dyes	
Table Settings	Table for 2 Alleles	
	Table for 10 Alleles	

*These templates must always be used for the data analysis. The other template files are optional.

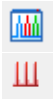
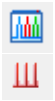
NOTE

Import of and allele calling with provided template files is only guaranteed if the GeneMapper™ ID-X software is used. When the GeneMapper™ software is applied you may experience import problems with some template files. You may have to adjust Panels and Bins with one or more runs of the allelic ladder on your specific instrument setup. Contact us for support (support@biotype.de).

Table 12 Data analysis workflow with GeneMapper™ ID-X

No	Icon	Working step
1		Software Preparation
		***Panel Manager*** Import the provided template files for Panel, Bins, Stutter
		***GeneMapper™ ID-X Manager*** Import the provided template for Analysis Method and Size Standard
2		Sample Import
		***Add Samples to Project*** - browse for run folder, select and ***Add to List → Add***
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	Select the previously imported BIOTYPE template AMLplexIVD_Analysis_v1x
Panel	Select the previously imported BIOTYPE template AMLplexIVD_Panel_v1x
Size Standard	Select the previously imported BIOTYPE template BTO_60-550_v1x

4		Check controls
		Check control validity (Allelic Ladder, Positive Control, Negative Control) With sufficient peak heights the assignment is carried out according to the specifications in the Analysis Method
5		Sample evaluation
		Check sample validity. With sufficient peak heights the assignment is carried out according to the specifications in the Analysis Method

NOTE

Using the provided template files for the Analysis Method, Bins, Panels, and selecting the corresponding sample type, the validity of these samples is checked by the software automatically. The quality control flags SOS (Sample Off-Scale), SQ (Sizing Quality), OMR (Outside Marker Range) shall be green boxes for a passed validity.

NOTE

Use the Size Match Editor in GeneMapper™ ID-X to evaluate the size standard. If an automatic fragment calling failed, the triplets 80 / 90 / 100 bp and 240 / 250 / 260 bp can be used for an orientation in manual peak assignment.

Troubleshooting

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

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. This effect should be taken into account in cases of high amplification profiles of the following targets:

- PML::RARA_bcr3 (yellow channel) caues pull-up peak in KMT2A::ELL_e10e2 (green channel)

- RUNX1::RUNX1T1 (blue channel) caues pull-up peak in KMT2A::MLLT4 (green channel, only if POP-7 ™ Polymer was used)

Template-independent addition of nucleotides

Because of its terminal transferase activity, the Multi Taq 2 DNA Polymerase tends to add an adenosine radical at the 3'-end of the amplified DNA fragments. The artefact peak is one base shorter than expected (-1 bp peaks). All BIOTYPE primers are designed to minimize these artefacts. Artefact formation is further reduced by the optional prolongation of the final extension step of the PCR protocol at 68 °C for 60 min. Peak height of the artefact correlates with the amount of cDNA. Laboratories should define their individual limits for analysis of the peaks.

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. If these effects occur, we recommend injecting the sample again at a higher room temperature. Always consider using fresh consumables according to the manufacturer's recommendations.

Influence of polymers

The Mentype® AMLplex^{QS} PCR Amplification Kit was validated and certified for the analysis on POP-4™ polymer. The use of other polymers (like POP-7™ or POP-1™) has been verified, but might influence the run behaviour of specific PCR products. Furthermore, background noise might increase through the different behaviour of free fluorescent dyes.

Pipetting deviations

The robustness analysis of the Mentype® AMLplex^{QS} PCR Amplification Kit showed that the kit is robust against minor deviations from the described protocol (+/- 10 % deviation). A medium deviation (+/- 20 %) from the described experimental protocol may be more critical for the analysis. It is essential to pay attention to medium deviations of - 20 % in PCR components, as they significantly reduce signal height. The most sensitive targets in this context are KMT2A::MLLT4 and DEK::NUP214.

To minimize any deviations, we recommend using calibrated pipettes, precise pipetting and thorough mixing.

Low ABL-Control peaks

The ABL internal control forms the longest amplicon of the Mentype® AMLplex^QS PCR Amplification Kit and is therefore an essential quality control marker. In case of low ABL-Control peak height, one of the following causes is the most likely:

Template input too low in the reaction: please determine the RNA concentration and increase cDNA synthesis input as necessary.

Fragmented RNA, i.e. smaller amplicons from targets may be positive, but the internal control ABL is missing. Check sample preparation workflow for RNase contamination. RNA fragmentation is known to occur in archived sample materials.

Ineffective cDNA synthesis: ensure compliance with manufacturers' instructions during the cDNA synthsis. Use one of the validated cDNA synthesis kits as described in chapter Transcription into cDNA.

Sample is compromised with active reverse transcriptase enzyme: ensure following the manufacturers' instructions with special regard to the heat-inactivation step during cDNA synthesis.

Additional amplicons or double-positives

The majority of patient samples is expected to show a negative result or positive result for one translocation. More than one translocation is an unsual result and may require further verification. However, some combinations have been observed in patient samples before such as the following:

The target BCR::ABL_b2a3 may appear with relatively low peak height if there is a strong amplification for the ABL-Control or any other transcript variant of BCR::ABL (about 5 - 10% of main target result).

The KMT2A partial tandem duplication (KMT2A-PTD) is known to occur simultaneously with other translocations and in complex rearrangements that may appear positive for more than one KMT2A-PTD variant.

When using POP-7™ polymer, the translocation CBFB::MYH11_Type A might lead to an unspecific signal for RUNX1::RUNX1T1 and

CBFB::MYH11_Type F. The RUNX1::RUNX1T1 peak is assigned as off ladder when using POP-4™ polymer and could be verified using the ME-1 cell line. Both unspecific side peaks show lower RFU peak height compared to CBFB::MYH11 Type A. When using POP-7™ polymer an unspecific off ladder peak may get assigned as translocation CBFB::MYH11_Type I with peak heights below 2000 RFU.

The target KMT2A::MLLT4 occurs with two different amplicon lengths depending on the sample specific prevalence of transcript variants. Most patient samples show both amplicons with a length difference of three nucleotides as a double peak in the KMT2A::MLLT4 target bin. This is a valid result, no corrective measures are necessary.

Any KMT2A translocation may occur with the breakpoint in exon 9 (most common) or exon 10 and 11 (rare). Any strong off ladder peak in the green channel may originate from an atypical KMT2A translocation and may be verified with other methods.

The target KMT2A::MLLT3 transcript variant 6A may produce two detectable amplicons in case of very high input and very high RNA quality, the corresponding peaks are 6A_S (short, required) and 6A_L (optional). This is a necessary phenomenon inherent in the Mentype® AMLplex^QS PCR Amplification Kit primer design. This is a valid result, no corrective measures are necessary.

An off ladder peak in the yellow channel between the bins of PML::RARA_bcr1 and _bcr3 is most likely caused by a variant of PML::RARA_bcr2. This target has several breakpoints in PML exon 6 and can therefore not be assigned with any specific bins.

Performance Evaluation

Analytical sensitivity

The sample type of the Mentype® AMLplex^QS PCR Amplification Kit is defined as cDNA transcribed from cellular RNA isolated from peripheral venous whole blood or bone marrow aspirate. For blood specimen RNA in the range from 50 ng to 1000 ng is measurable with sufficient peak height of the ABL-Control using the during this performance evaluation validated cDNA synthesis kits SuperScript™ IV First-Strand Synthesis System (Thermo

Fisher Scientific) and High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Assuming a 100 % transcription efficiency this results in a cDNA concentration range of 2.5 ng/µL to 50 ng/µL; 1 µL of cDNA is used in the PCR reaction. For bone marrow specimen RNA in the range from 50 ng to 1000 ng using the SuperScript™ IV First-Strand Synthesis System (Thermo Fisher Scientific) and from 100 ng to 1000 ng using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) is measurable with sufficient peak height of the ABL-Control. Assuming a 100 % transcription efficiency this results in a cDNA concentration range of 2.5 ng/µL respectively 5 ng/µL to 50 ng/µL; 1 µL of cDNA is used in the PCR reaction. The optimal RNA input is defined as 1000 ng for peripheral venous whole blood specimen and 500 ng for bone marrow specimen. In case of samples with poor RNA concentration or critical targets, the cDNA input in the PCR can be increased up to 4 µL for cDNA transcribed with the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), regardless the specimen type. For cDNA transcribed with the SuperScript™ IV First-Strand Synthesis System (Thermo Fisher Scientific), the cDNA input is limited to a maximum of 2 µL.

The Limit of Blank (LoB) was tested on 12 samples with different specimen origin at the previously defined optimal input amount. As no unspecific peaks were detected above the threshold of 400 RFU peak height in the range of 55 bp to 550 bp in any of the 12 tested samples, a threshold of 400 RFU can be set.

The Limit of Detection (LoD) of the Mentype® AMLplex^{QS} PCR Amplification Kit was tested with 6 artificial samples with cDNA background, covering all primer pairs in the primer mix with primer pairs detecting more than one target amplifying the longest and therefore hardest to detect target transcript variant.

The Mentype® AMLplex^{QS} PCR Amplification Kit showed an acceptable Limit of Detection according to the acceptance criteria with a maximum LoD of 400 copies for the targets tested (see Table 13), except for the KMT2A::MLLT4, which still showed an acceptable result with a LoD of 1000 copies. Since not all transcript variants of the gene fusions detectable could be tested, an overall LoD of 1000 copies, which was the highest result of the targets tested, is assumed to be safely applicable.

Table 13. LoD for the Mentype® AMLplex^QS PCR Amplification Kit.

Sample	Target	LoD [copies]
Sample 1	CBFB::MYH11_TypeJ	200
	BCR::ABL_e1a2	200
	CBFB::MYH11_TypeB	200
	KMT2A-PTD_e11e3	200
	PML::RARA_bcr1	400
Sample 2	CBFB::MYH11_TypeC	200
	CBFB::MYH11_TypeA	100
	NPM1::MLF1	200
	KMT2A::MLLT3_6A_S	100
	KMT2A::MLLT3_6A_L	200
	PML::RARA_bcr3	200
Sample 3	CBFB::MYH11_TypeG	200
	BCR::ABL_b2a3	100
	CBFB::MYH11_TypeE	200
	KMT2A::ELL_e10e3	100
Sample 4	CBFB::MYH11_TypeI	200
	MLLT10_240::PICALM_2092	200
	RUNX1::RUNX1T1	400
	DEK::NUP214	200
Sample 5	KMT2A::MLLT4 (short)	1000
Sample 6	KMT2A::MLLT4 (short)	500
	KMT2A::MLLT4 (long)	500

As the Mentype® AMLplex^QS PCR Amplification Kit is a qualitative assay designed to determine the presence or absence of the target analyte, testing the Limit of Quantitation (LoQ) is not considered relevant for the intended use.

Analytical Specificity

We tested the automatic allele calling with the allelic ladder and the concordance of the allele assignment compared to artificial samples of the translocation targets using the GeneMapper™ ID-X software. Based on the results, the test-specific device settings for genotyping by means of capillary gel electrophoresis (bins and panels) of the Genetic Analyzer are defined.

PCR primers are designed to bind complementary to the official human genome reference sequence. Genetic variants such as Single Nucleotide Polymorphism (SNPs, INDELs or deletions) may affect specific primer binding and were assessed by entries of the dbSNP relevant for the Mentype® AMLplex^{QS} PCR Amplification Kit. One entry (rs73504425) results in loss of primer function. The variant allele occurs in 0.6 % of the African population and locates within the primer binding site for PICALM::MLLT10, a rarely reported fusion gene with a prevalence < 1 %.

Futhermore, a BLAST search was performed on the human transcriptome for the Mentype® AMLplex^{QS} Primer mix and yielded no unspecific amplification products in the range of 0 – 600 bp.

Accuracy and Trueness

The analytical accuracy of the Mentype® AMLplex^{QS} PCR Amplification Kit is based on the results of trueness studies of the kit. BIOTYPE GmbH actively participates in an External Quality Assessment (EQA) program since 2013. The presence or absence of a translocation within the samples is determined from the consensus of the results of all participants of this EQA. In total 68 samples were analyzed since 2013.

Based on these results a contingency matrix was created based on the comparison of the Mentype® AMLplex^{QS} PCR Amplification Kit results (positive/negative) and the true results (positive/negative), confirmed by the consensus of all participants of the External Quality Assessment program. According to CLSI EP12 (3rd edition) the key performance metrics shown in Table 14 were calculated based on the contingency matrix.

Table 14 Analytical characteristics of the Mentype® AMLplex^{QS} PCR Amplification Kit.

Analytical Characteristics	Estimate	Lower 95 % Confidence Interval	Upper 95 % Confidence Interval
Analytical Sensitivity	92.2 %	81.5 %	96.9 %
Analytical Specificity	94.1 %	73.0 %	99.0 %
Positive Predictive Value	97.9 %	89.1 %	99.6 %
Negative Predictive Value	80.0 %	58.4 %	91.9 %
Accuracy	92.6 %	83.9 %	96.8 %

These calculations provide a comprehensive measure of the Mentype® AMLplex^{QS} PCR Amplification Kit's ability to correctly classify positive and negative cases with 92 % and 94 % probability respectively. The overall proportion of correct results is 93 %, showing a high reliability. The Mentype® AMLplex^{QS} PCR Amplification Kit demonstrates high analytical performance, validating the assay's effectiveness and reliability.

Precision

We evaluated the assay repeatability and reproducibility based on the ISO 5725-2:2022-05 and the CLSI EP05 (3rd edition). Five artificial samples and the Mentype® AMLplex^{QS} Positive Control were evaluated in a 5 x 5 x 3 (day x replicate x site) multisite study. The multisite study examines the main source of variation (repeatability and reproducibility) due to the different tested sites which will present different operators measuring at different sites using different instruments. The resulting repeatability ranged between 8.3 %CV and 15.6 %CV and reproducibility ranged between 14.6 %CV and 30.5 %CV. Overall, the Mentype® AMLplex^{QS} PCR Amplification Kit shows an acceptable precision with $SD_{Rep} \leq 2500$ RFU for all specific gene fusions tested with the artificial Samples 1 to 5 and for the Mentype® AMLplex^{QS} Positive Control.

Assay Cut-Off

The Mentype® AMLplex^{QS} PCR Amplification Kit uses qualitative detection of gene fusions by endpoint PCR. As such, one of the relevant cut-offs for the assay is the minimum peak height to safely distinguish between technical background noise and genuine target detection. RFU peak height cut-off was determined at 400 RFU with data from the analytical sensitivity testing.

Interferents and cross-reactions

Potential interferents which could influence the results of the measurement procedure were evaluated in line with the recommendations of the CLSI guidelines EP07 (3rd edition) and EP37 (1st edition). For the Mentype® AMLplex^{QS} PCR Amplification Kit, the endogenous and exogenous interferents were determined, their maximum expected concentration (C_{max}) in the PCR reaction, as suggested in the guidelines, was tested, and in case interference was observed, further concentrations were tested to determine the concentration at which no interference is detectable.

Results showed no interfering effect of the endogenous interferent DNA tested at 1 ng in the PCR reaction, when DNA digestion was carried out as part of sample processing.

As for the endogenous interferent blood an interfering effect was observed at the calculated C_{max} of 0.87 % v/v in the PCR reaction, further dilutions were tested until no interference was detectable. 0.087 % v/v of blood showed no interference. However, blood contamination can be visually detected in the PCR reaction or in the isolated RNA suspension by an orange color shift. If a color change is observed, we would recommend repeating the RNA isolation to avoid any potential interference.

For the exogenous interferents DTT, RNase Inhibitor, DNase, Ethanol, Metoclopramide, EDTA, Hydroxyurea and Citrate no interference effect was observed at their respective C_{max}, where deviations from the untreated group observed for the tested samples were within the accepted limits.

As for the exogenous interferents Reverse Transcriptase, Heparin and Proteinase K interference was observed at the respective C_{max} calculated or recommended by CLSI EP37 (1st edition), further dilutions were tested until no interference was detectable. For the enzyme Reverse Transcriptase the

tested C_{max} 0.2 % v/v showed interference unless the enzyme is heat inactivated as instructed by the manufacturers of tested cDNA synthesis kits. For Heparin 3.3 U/dL and for the enzyme Proteinase K 0.0001 % v/v showed no interference.

The blood anticoagulants EDTA, sodium citrate and heparin were additionally tested with the isolation kits recommended. No impact on the analytical performance was detected. We anticipate that the workflow of the isolation kits will effectively remove any tested interfering substances derived from anticoagulants and RNA isolation reagents.

Table 15 Tested non-interfering concentrations of endogenous and exogenous interferents.

Type of Interferent	Category	Interferent	Non-interfering tested concentration
Endogenous	Whole blood components	Whole blood	0.087 % v/v in PCR reaction
Exogenous	Anticoagulants	EDTA	0.099 mg/dL
		Citrate	0.004 % v/v in PCR reaction
		Heparin	3.3 U/dL
	RNA isolation reagents	Proteinase K	0.0001 % v/v in PCR reaction
		Ethanol	0.3 % v/v in PCR reaction
		DNase	0.16 % v/v in PCR reaction
	cDNA reverse transcription reagents	DTT	0.2 % v/v in PCR reaction
		RNase Inhibitor	0.2 % v/v in PCR reaction
		Reverse Transcriptase	0.2 % v/v in PCR reaction **(heat inactivated)**
	Antiemetic agent	Metoclopramide	0.225 mg/dL
	Antineoplastic agent	Hydroxyurea	3.08 mg/dL

In-Use Stability

All stability studies were planned conformant with ISO 23640:2015 and the CLSI EP25 (2nd edition) guideline. The following procedure was conducted for all stability studies: The Mentype® AMLplex^{QS} PCR Amplification Kit was tested at multiple timepoints over various durations. Artificial samples and the Mentype® AMLplex^{QS} Positive Control were analyzed. The final evaluation of the various conditions included the comparison between the means of the starting timepoint (T_0) and the subsequent timepoints (T_n) and was calculated using the following equation:

$$abs.\Delta_n = |\overline{x}_{T0} - \overline{x}_{Tn}|$$

For the in-use stability study, two experiments were performed. One to test the stability after several freeze and thaw cycles and one to test the stability of the kits during simulated use after opening as part of the freeze and thaw and claimed shelf life stability study.

Based on the results, the Mentype® AMLplex^{QS} PCR Amplification Kit is stable for up to 20 freeze and thaw cycles, and can be used for up to 24 months after opening.

Clinical performance data

Study design, ethics and regulatory aspects

The Mentype® AMLplex^{QS} PCR Amplification Kit and reference methods were tested on 297 patient samples and 10 healthy volunteers. The aim of this study was to provide clinical evidence according to §§20 to 24 of the medical device act 'Medizinproduktgesetz'. Using the reference methods cytogenetic (FISH) and or internal validated monoplex-qPCR a concordance with the device had to be proven. The confirmation of the responsible ethic commission was received on 06.06.2012.

Reference methods

The primary target is the determination of diagnostic sensitivity and specificity in comparison to reference methods. As reference method, standardized Fluorescence-In-Situ-Hybridization (FISH) was conducted for a selection of translocations [Grimwade et al. Blood 116: 354-65, 2010].

Translocations not addressable with cytogenetics were tested with monoplex-nested-PCR-tests established and validated in the test laboratory [Steudel *et al.* Genes Chromosomes Cancer 37: 237-51, 2003, van Dongen *et al.* Leukemia 13: 1901-28, 1999].

RNA extraction and purification

Mononuclear cells (MNC) were collected from the samples with density gradient centrifugation. Subsequently, total RNA isolation and reverse transcription to cDNA were performed using commercially available kits. The quality of the cDNA was analyzed through real-time PCR. Validated single PCR assays served to confirm the fusion genes and mutations.

Results

The Mentype® AMLplex^{QS} PCR Amplification Kit showed no false positive results with the tested cDNA of the 10 healthy volunteers.

Out of 297 tested patient samples, 5 could not be evaluated due to detection of the internal ABL-Control below the recommended threshold. Of the remaining 292 samples, 201 showed true negative results in comparison to the reference methods.

The individual results of the comparison tests are summarized in Table 16. Gene fusions PICALM::MLLT10 (CALM-AF10) and KMT2A::MLLT4 (MLL-AF6) could not be evaluated with regard to diagnostic sensitivity, as no positive pre-characterized samples were available.

Overall, a diagnostic sensitivity of 94 % and a diagnostic specificity of 99.5 % were achieved.

Table 16 Conclusion of the clinical performance data results of the Mentype® AMLplex^QS PCR Amplification Kit. *Prevalence data was cited from Grimwade et al. 2010. n.e. = not evaluated, chrom. = chromosal, diagn. = diagnostic, Prev = prevalence

Biomarker			Evaluation of clinical performance (n = 292)						
Gene Fusion	Chrom. aberration	Variant	Prev* [%]	True positive	True negative	False postive	False negative	Diagn. Sensitivity [%]	Diagn. specificity [%]
RUNX1:: RUNX1T1	t(8;21) (q22;q22)	n. b.	7	16	275	0	1	94.1	100.0
BCR::ABL	t(9;22)	e1a3	1	1	291	0	0	100.0	100.0
	(q34;q11)	e1a2							
		b3a2							
		b3a3							
		b2a2							
		b2a3							
PICALM::MLLT10	t(10;11) (p13;q14)	MLLT10_240-PICALM_1987	1	0	292	0	0	n.e.	100.0
		MLLT10_240-PICALM_2092							
CBFB:: MYH11	inv(16)	Type A	5	28	262	0	2	93.3	100.0
	(p13;q22)	Type B							
		Type C							
		Type D							
		Type E							
		Type F							
		Type G							
		Type H							
		Type J							
DEK:: NUP214	t(6;9) (p23;q34)	n. b.	1	3	289	0	0	100.0	100.0
KMT2A:: MLLT4	t(6;11) (q27;q23)	n. b.	<0,5	0	292	0	0	n.e.	100.0
KMT2A:: MLLT3	t(9;11)	6A (6A_S; 6A_L)	1	4	287	0	1	80.0	100.0
	(p22;q23)	7A							
		8A							
		6B							
KMT2A:: ELL	t(11;19)	e10e2	1	0	290	1	1	0.0	99.7
	(q23;p13.1)	e10e3							
KMT2A-PTD	Partial	e9e3	5-7	23	269	0	0	100.0	100.0
	Tandem	e10e3							
	Duplication	e11e3							
NPM1:: MLF1	t(3;5) (q25.1;q34)	n. b.	<0,5	2	290	0	0	100.0	100.0
PML:: RARA	t(15;17)	bcr1	13	8	284	0	0	100.0	100.0
	(q22;q21)	bcr2							
		bcr3							
Summary			37	**85**	**201**	**1**	**5**	**94.4**	**99.5**

Diagnostic Evaluation

The clinical performance characteristics of the Mentype® AMLplex^QS PCR Amplification Kit showed acceptable results. The parameters for clinical performance evaluation were calculated according to Annex I, Sec. 9.1b of IVDR (EU) 2017/746 as displayed in Table 17.

Table 17 Diagnostic characteristics of the Mentype® AMLplex^QS PCR Amplification Kit

Diagnostic Characteristic	Estimate	Lower Confidence Interval	Upper Confidence Interval
Diagnostic Sensitivity	94.4 %	87.7 %	97.6 %
Diagnostic Specificity	99.5 %	97.3 %	99.9 %
Positive Predictive Value	98.8 %	93.7 %	99.8 %
Negative Predictive Value	97.6 %	94.4 %	99.0 %
Diagnostic Accuracy	98.0 %	95.6 %	99.1 %

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

Asou H, Tashiro S, Hamamoto K, Otsuji A, Kita K, Kamada N (1991)
Establishment of a human acute myeloid leukemia cell line (Kasumi-1) with 8;21 chromosome translocation. Blood 77(9): 2031-2036.

Beillard E, Pallisgaard N, van der Velden VHJ, Bi W, Dee R, van der Schoot E, Delabesse E, Macintyre E, Gottardi E, Saglio G, Watzinger F, Lion T, van Dongen JJM, Hokland P, Gabert J (2003)
Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using ‚real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR)- a Europe against cancer program. Leukemia 17:2474-2486.

Schnittger S, Kinkelin, Schoch U, Heinecke, A, Haase D, Haferlach T, Büchner T, Wörmann B, Hiddemann W & Griesinger F (2000) Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 14, 796–804

Van Dongen JJM, Macintyre EA, Gabert JA, Delabesse E, Rossi V, Saglio G, Gottardi E, Rambaldi A, DOtti G, Griesinger F, Parreira A, Gameiro P, Gonzalez Diaz M, Malec M, Langerak AW, San Miguel JF, Biondi A (1999)
Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease - Report of the BIOMED-1 Concerted Action: Investigation of minimal residual disease in acute leukemia. Leukemia 13:1901-1928.

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 validated for use with the reagents, instruments and software described in chapter Material and devices required but not provided.
- The Mentype® AMLplex^{QS} PCR Amplification Kit has been designed, validated, and certified as a screening tool for the subtype classification of AML. This application is not suited to quantify copy numbers like monitoring Minimal Residual Disease (MRD) or to validate other subtypes of leukemias or pediatric AML.
- Due to genetic variability, single nucleotide polymorphism (SNPs) or short insertion deletion polymorphism (INDELs) may affect primer efficacy or template accessibility. An analysis of the most recent version of the human genome assembly (HG38) for all ethnic groups found only one critical SNP that results in loss of primer function. The variant allele occurs in 0.6 % of the African population and locates within the primer binding site for PICALM::MLLT10, a rarely reported fusion gene with a prevalence < 1 %. However, due to the low gene fusion prevalence in combination with low likelihood of SNP occurrence in the worlds' population, the risk of false negative results is low. In case of any uncertainties, please consider confirming results using reference methods.
- 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® AMLplex^QS PCR Amplification Kit	25 reactions	45-12100-0025
	100 reactions	45-12100-0100
	400 reactions	45-12100-0400
Matrix Standard BT5 multi	1 x 25 µL	45-15100-0025
	2 x 25 µL	45-15100-0050

Trademarks and Disclaimers

Mentype® is a registered trademark of BIOTYPE GmbH.

Other trademarks: ABI PRISM®, GeneMapper™, SuperScript™ GeneAmp® and Applied Biosystems® (Applied Biosystems LLC group); QIAamp® (Qiagen); POP-4™, POP-1™,POP-7™ (Europe: Applied Biosystems LLC, US: Life Technologies Corporation). The PCR is covered by patents. Patentees are Hoffmann-La Roche Inc. and F. Hoffmann-La Roche (Roche).

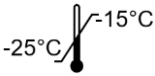
Registered names, trademarks, etc. used in this document, even if not specifically marked as such, are not to be considered unprotected by law.

The Mentype® AMLplex^QS PCR Amplification Kit is a CE-marked diagnostic kit according to the European in vitro diagnostic regulation (EU) 2017/746.

Product not licensed with Health Canada and not FDA cleared or approved.

Not available in all countries.

Explanation of Symbols

	Manufacturer
	Batch code
	Contains sufficient reagents for <N> tests
	Consult electronic instructions for use (eIFU)
	Use-by date
	Temperature limit
	Catalogue number
	In vitro diagnostic medical device
	Keep away from sunlight
	Keep dry
	Unique device identifier

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
indented, cursive, bold text	Fields which are to be clicked in a software

Appendix

Target gene list

Gene	Alternative names	Transcript ID
RUNX1	AML1, AMLCR1, CBFA2, PEBP2A2	ENST00000675419.1
RUNX1T1	AML1T1, CBFA2T1, CDR, ETO, MTG8, ZMYND2	ENST00000523629.7
BCR	ALL, BCR1, CML, D22S11, D22S662, PHL	ENST00000305877.13
ABL1	ABL, C-ABL, JTK7, P150	ENST00000318560.6
MLLT10	AF10	ENST00000307729.12
PICALM	CALM, CLTH	ENST00000393346.8
CBFB	PEBP2B	ENST00000412916.7
MYH11	SMHC, SMMHC, SMMS-1	ENST00000300036.6
DEK	D6S231E	ENST00000652689.1
NUP214	CAIN, CAN, D9S46E, N214	ENST00000359428.10
KMT2A	ALL-1, ALL1, CXXC7, HRX, HTRX, HTRX1, MLL, MLL1, MLL1A, TRX1	ENST00000534358.8
MLLT4	AF-6, AF6, AFDN	ENST00000683244.1
MLLT3	AF-9, AF9, YEATS3	ENST00000380338.9
ELL	C19ORF17, ELL1, MEN, PPP1R68	ENST00000262809.9
NPM1	B23, NPM	ENST00000296930.10
MLF1	-	ENST00000466246.7
PML	MYL, RNF71, TRIM19	ENST00000268058.8
RARA	NR1B1, RAR, RAR-ALPHA, RARALPHA	ENST00000254066.10

Electropherograms of reference samples

On the following pages you can find examples of the electropherograms of the Mentype® AMLplex^{QS} Allelic Ladder (Figure 2), the Mentype® AMLplex^{QS} Positive Control (PC, Figure 3) and a no template control (NTC, Figure 4).

All samples were amplified on a ProFlex PCR cycler and analyzed on a 3500 Genetic Analyzer (POP-4™, 36 cm array) using the validated run parameter. The data analysis was performed using GeneMapper™ ID-X version 1.6. Bins, Panels, and Analysis method according to Table 11 were applied.

The electropherograms are zoomed to a fragment length of 50 – 560 bp (x-axis). The scaling of the y-axis was performed individually:

Figure 2, page 55: Mentype® AMLplex^{QS} Allelic Ladder: 3,000 RFU

Figure 3, page 56: Mentype® AMLplex^{QS} Positive Control: 9,000 RFU

Figure 4, page 57: No template control (NTC): 9,000 RFU

Mentype® AMLplex^QS Allelic Ladder

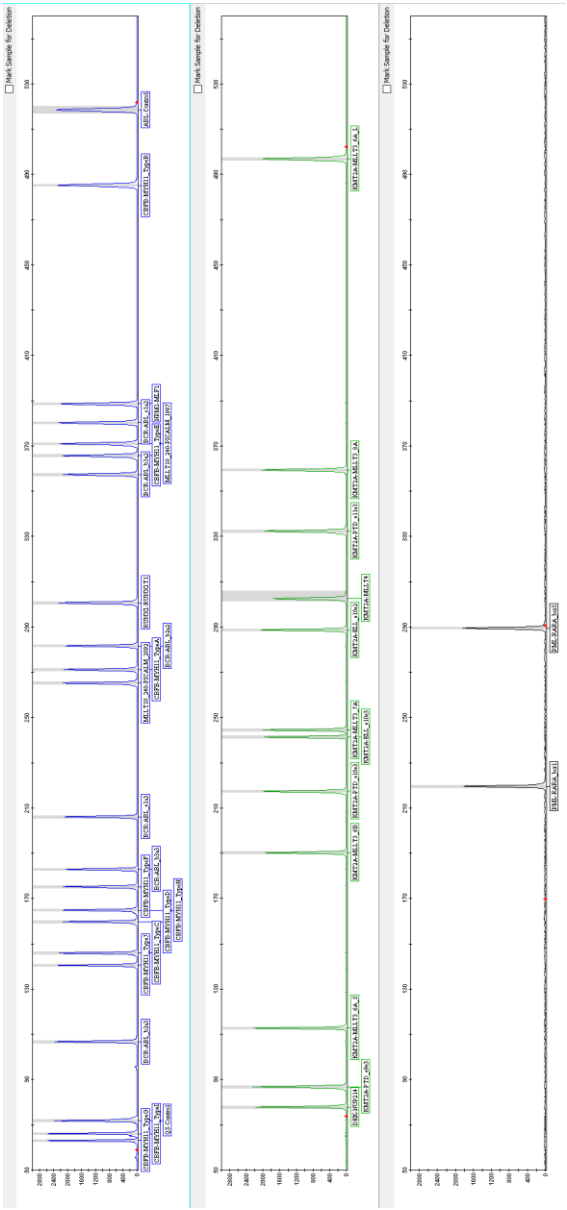


Figure 2 Mentype® AMLplex^QS Allelic Ladder

Mentype® AMLplex^QS Positive Control (PC)

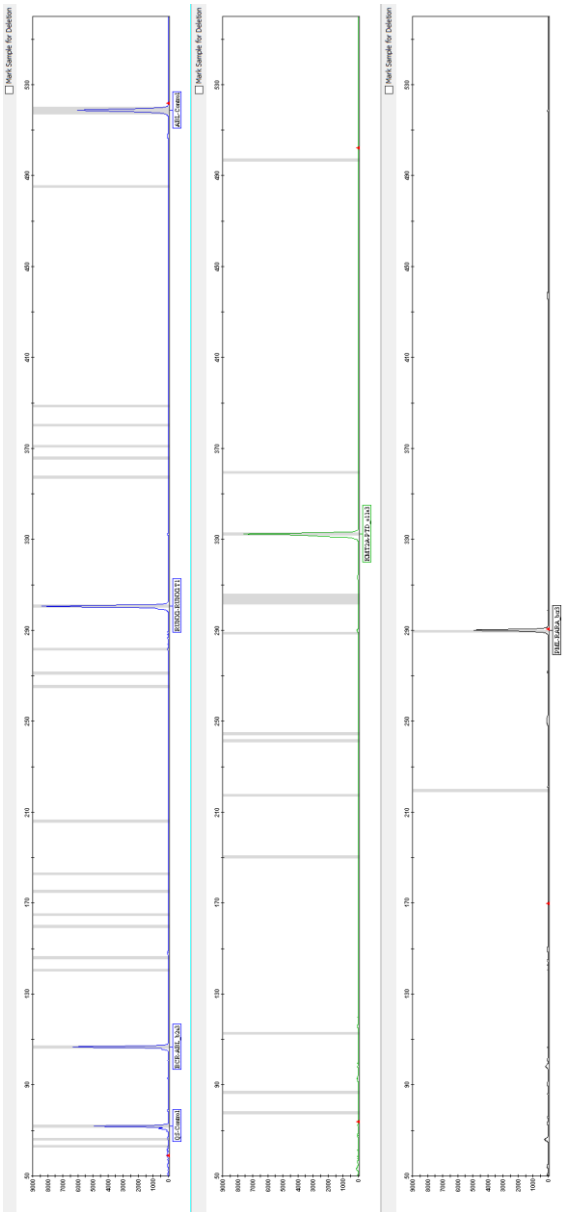


Figure 3 Mentype® AMLplex^QS Positive Control (PC)

No template control (NTC)

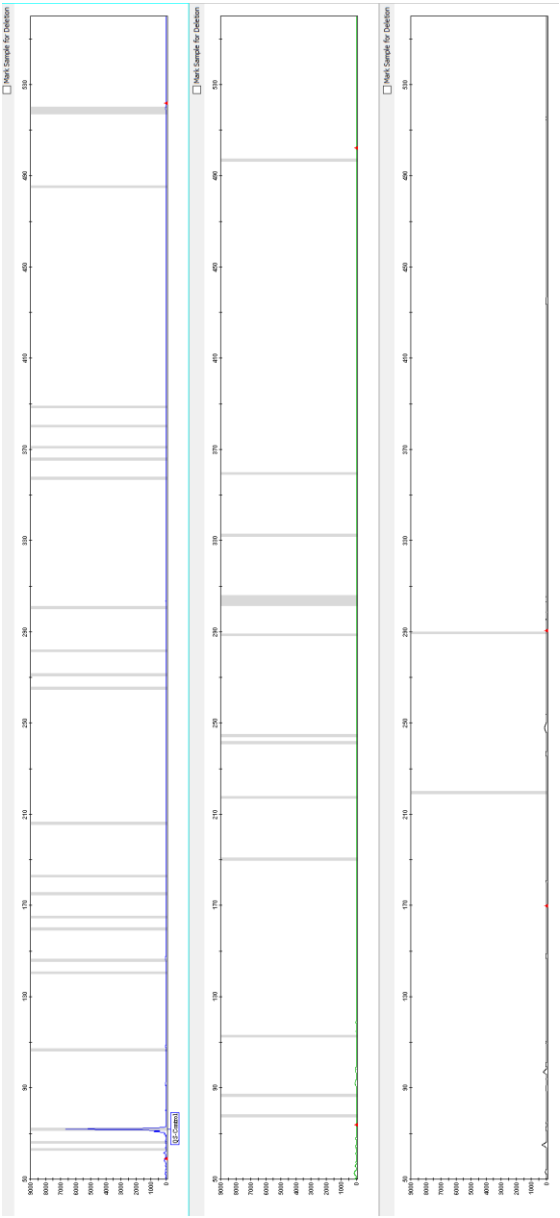


Figure 4 No Template Control (NTC)

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