Matrix Standard BT5 multi

Instructions for Use (IFU)

CE IVD For in vitro diagnostic use

MSTIFU02v4en
July 2026

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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 IFU version:

Document code	Changes	Date
MSTIFU02v1en	Initial version	26.03.2025
MSTIFU02v2en	Change of ordering number Mentype® DIPscreen PCR Amplification Kit	30.04.2025
MSTIFU02v3en	Change of ordering number Mentype® AMLplex^{QS} PCR Amplification Kit	10.06.2025
MSTIFU02v4en	Note on device preparation for other products	31.07.2026

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 Matrix Standard BT5 multi is a reagent used to manually generate a matrix for the spectral alignment of the Applied Biosystems® 3500 and SeqStudio™ Genetic Analyzer (Thermo Fisher Scientific). It is required to analyze PCR products of the IVD products Mentype® AMLplex^{QS} PCR Amplification Kit, Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit. The matrix is used by the device software to automatically analyze the five different fluorescent signals of the PCR products in one capillary.

The Matrix Standard BT5 multi is intended for professional laboratory users trained on molecular-genetic techniques, multiplex PCR, and the handling of Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division).

Scientific background

The Matrix Standard BT5 multi is a mixture of five PCR amplicons, each labeled with one of the five fluorophores 6-FAM™, BTG, BTY, BTO, BTR. By analyzing these oligonucleotides, the spectral overlap between the emission spectra of the dyes is calculated and corrected, enabling the simultaneous analysis of all five dyes in one capillary. This leads to a reduction of artefacts such as pull-up peaks and enables the data analysis of PCR fragments from the PCR amplification kits Mentype® Chimera®, Mentype® DIPscreen and Mentype® AMLplex^{QS}.

NOTE

Other BIOTYPE products based on the fluorophore combination 6-FAM™, BTG, BTY, BTO, and BTR may require device preparation with the Matrix Standard BT5 multi prior to analysis. Corresponding information can be found in the respective product-specific handbook or instructions for use.

Materials provided

Kit content

Table 1 Matrix Standard BT5 multi kit content

Reagent	Cap color		Volume per packaging size	
			25 reactions	50 reactions
Matrix Standard BT5 multi	Black		25 µL	2 x 25 µL

Description of components

The Matrix Standard BT5 multi contains five oligonucleotides labeled with the fluorophores 6-FAM™, BTG, BTY, BTO and BTR. The oligonucleotides are premixed in one vial. The product is prepared with a volume of 25 µL and several vials are included to suit the kit reaction size.

NOTE

Synthetic oligonucleotides are not critical for the intended user, as these consist 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. Please also refer to the product's non-hazardous statement (NHS), that is available on request.

NOTE

Please note that the packaging size describes the number of tests or the number of capillaries to be tested. Please take into account the required excess for pipetting.

Reagent storage and handling

The kit is shipped on dry ice. The components of the kit should arrive frozen. Please check the completeness of the kit upon receipt. Do not use kits that have been thawed upon arrival. If the included component is not frozen, or if tubes or the packaging have been compromised during the shipment, the performance cannot be guaranteed.

Store all components at -25 °C to -15 °C, protected from light. In order to prevent contamination, we recommend to store the reagents in a post-PCR area, seperated from pre-amplification reagents.

Materials and devices required but not provided

General laboratory equipment

- 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
- Disposable powder-free gloves

Reagents, kits and consumables

Table 2 Reagents required, but not provided

Reagent	Supplier	Order number
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

The Matrix Standard BT5 multi was initially validated on the Applied Biosystems® 3130 Genetic Analyzer, however, as the device has been discontinued, these are the instruments that are verified. The corresponding consumables are listed in Table 2.

- Applied Biosystems® 3500 Genetic Analyzer (Thermo Fisher Scientific), software version 4.0.1
- SeqStudio™ Genetic Analyzer (Thermo Fisher Scientifc), software version 1.1.4 and version 1.2.4

The matrix evaluation was performed using the device specific software as mentioned above.

NOTE

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

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. For products that do not require an SDS as they do not contain an SVHC or are subject to other restrictions of Regulation 1272/2008 (CLP), BIOTYPE provides the SDS/NHS upon request.

- Please contact the respective manufacturers for copies of the SDS for any additionally needed reagents.
- Components of different kit lots must not be mixed.
- Do not add any reagents other than the Matrix Standard BT5 multi to the denaturation mixture.
- 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
- Do not use a kit that has passed its expiration date.
- Discard kit waste according to local laws and regulations.
- All instruments used must have been installed, calibrated, checked, and maintained according to the manufacturer´s instructions and recommendations.

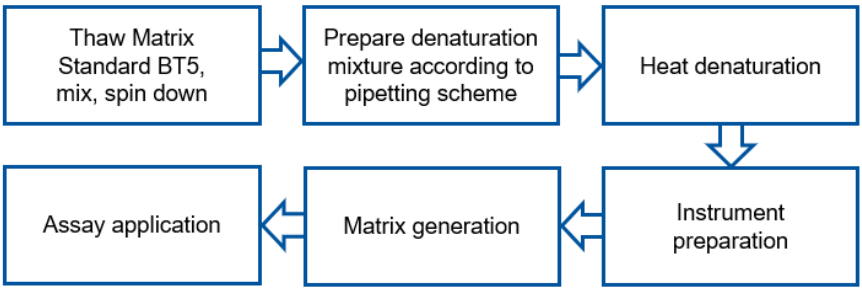
Notice to the user

Any problem that occurs in relation to the product shall be reported by the user to the manufacturer. Any serious incidents related with 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.

As the Matrix Standard BT5 multi is classified as a class A product, according to rule 5 of Annex VIII IVDR no Summary of Safety and Performance (SSP) is required. The accessory is used as a mandatory prerequisite in order to analyze PCR products from the IVDR-compliant kits Mentype® Chimera® PCR Amplification Kit, Mentype® DIPscreen PCR Amplification Kit and Mentype® AMLplex^{QS} PCR Amplification Kit. For more information about the safety of the aforementioned products in accordance with Article 29 of Regulation (EU) 2017/746, please refer to the assay-specific SSPs, publicly available via EUDAMED database.

Procedure

Overview of the experimental workflow



When to perform a spectral alignment

NOTE

Please refer to the manual of your Genetic Analyzer and the manufacturer's recommendations for the performance of a ***spectral calibration***.

- A dye is first used on the Genetic Analyzer
- The capillary array, capillary array length or polymer type is changed
- The instrument is moved or the detection cell door is opened
- The laser/CCD camera have been realigned/replaced during service
- An atypical increase in (pull-up and/or pull-down peaks) is observed

Preparation of denaturation mixture

The reagent Matrix Standard BT5 multi must be thawed at room temperature (22 °C to 25 °C, protected from light) then homogenized by vortexing. After this, briefly centrifuge the reagent (approx. 5 s) and prepare the following

denaturation mixture according to the number of capillaries (cap.) of the Genetic Analyzer (Thermo Fisher Scientific) as recommended in Table 3.

Table 3 Pipetting scheme according to capillary number (cap.)

	Volume per component [µL]				
Material	**4 cap.**	**8 cap.**	**24 cap.***	**48 cap.***	**96 cap.***
Hi-Di™ Formamide	60.0	108.0	300.0	600.0	1200.0
Matrix Standard BT5 multi	5.0	9.0	25.0	50.0	100.0
Total volume	65.0	117.0	325.0	650.0	1300.0
Genetic Analyzer	SeqStudio	3500	3500XL	3730	3730XL

* The application on 24, 48 and 96 capillary instruments has not been validated.

NOTE

Please note that the matrix generation of one 48-capillary device needs at least the 45-15100-0050 packaging size of the product, and two times for a 96-capillary device.

Pipette 12.0 µL of the denaturation mixture into the wells of a PCR plate (suitable for use with the Genetic Analyzer) at the corresponding plate position as defined in the ***spectral calibration wizard*** of the Genetic Analyzer. Spin down briefly.

NOTE

To uphold the principles of good laboratory practice, it is advisable to handle formamide with proper body, hand and eye protection.

Heat denaturation

Place the PCR plate in a standard thermal cycler and use the following protocol for an additional heat denaturation:

Table 4 Heat denaturation protocol

Temperature	Time
95 °C	3 min
10 °C	∞

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.

Instrument preparation

NOTE

Follow the instrument manufacturer's instructions for use to create instrument protocols.

1. Prepare a custom dye set by using the ***AnyDye*** Template for SeqStudio™ or 3500 Genetic Analyzers.
2. Assign the calibration peak order to:
   5-blue, 4-green, 3-yellow, 2-red, 1-orange.

   Set the ***Condition number*** **to 15** and the ***Q-value*** **to 0.95**. All other parameters must remain default.
3. Enter a name (e. g. BT5)

Matrix generation

NOTE

Follow the instructions according to the ***spectral calibration wizard*** of your Genetic Analyzer. Please refer to the manufacturer's manual.

NOTE

Before starting the spectral alignment, it is recommended to use the ***Fill the array with fresh polymer*** wizard in order to rinse all capillaries and reduce the risk of artefact formation. Please also refer to chapter Troubleshooting case of failed calibrations.

1. Start the ***spectral calibration*** according to the manufacturer's instructions and according to the plate position used. **Disable** the option ***Allow borrowing***.

During the spectral alignment, the instruments' software verifies the acceptance criteria in each capillary according to the dye set protocol.

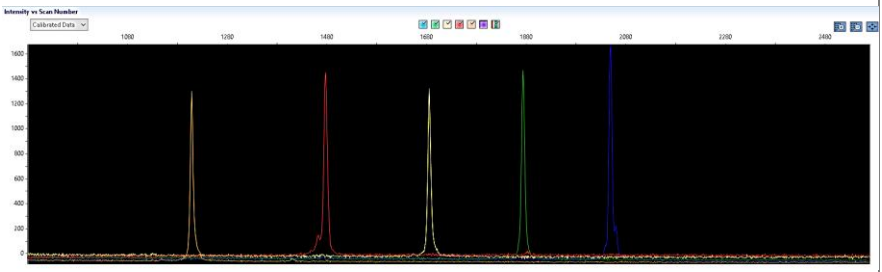
The quality and validity of each capillary is displayed in the data collection software of the Genetic Analyzer and in the respective calibration report (passed or failed).

2. After the spectral alignment check the ***Quality Value*** (Q Value) that should be greater than **0.95** and the ***Condition number*** that should range between **1** and **15.**

   Both values can be found in the ***calibration report*** (see Figure 1 for 3500 Genetic Analyzer (B) and SeqStudio™ Genetic Analyzer (C))

NOTE

Please consider storing the calibration reports. They can prove beneficial in case of any discrepancies or if support is required.

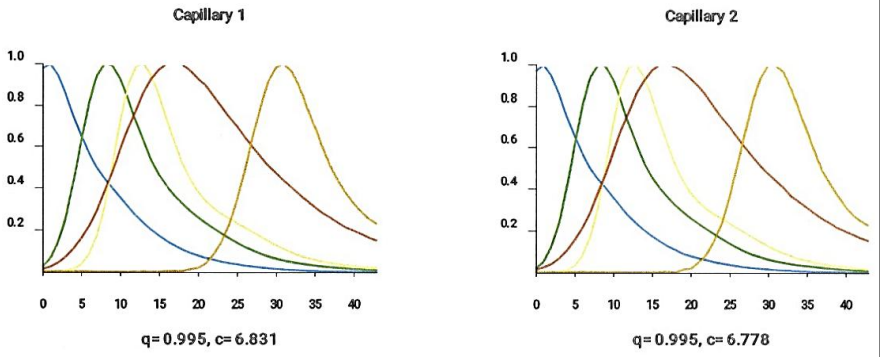


A. Calibration plot e. g. ***Intensity vs. scan number*** on 3500 Genetic Analyzer. View from data collection software.

Capillary	1	2	3	4	5	6	7	8
Run 1								
Run 2								
Run 3								
Overall								

Legend: Passed Failed Borrowed Not Calibrated

B. Extract from the calibration report of the 3500 Genetic Analyzer. All passed capillaries appear green.



C. Calibration plot ***Intensity vs. pixel number*** from calibration report on SeqStudio™ Genetic Analyzer with 2 exemplary capillaries

Figure 1. Visualization of calibration data on different Genetic Analyzers

Troubleshooting

NOTE

For technical advice regarding the Matrix Standard BT5 multi please contact BIOTYPE's Customer Support via support@biotype.de

For general instructions how to perform ***spectral calibrations*** on Genetic Analyzers please refer to the manufacturer's manuals.

- If an insufficient number of dye spectra have been detected please ensure that the denaturation mixtures have been properly vortexted before. Do not use expired or incorrectly stored reagents.
- The correct dye peak order in the dye set protocol is crucial for a successful spectral alignment. If the procedure failed, please check respective settings as described in chapter Instrument preparation.
- Always check the raw data of the subsequent assay-specific results in order to find spectral inconsistencies like pull-up or pull-down peaks. If such artefacts are interfering with the data analysis of the assay performed, please repeat the matrix generation. Please refer to chapter When to perform a spectral alignment.
- If an unsteady baseline or artefacts are interfering with the matrix generation, please repeat the injection using fresh consumables and materials. Also consider possible contaminants interfering with the analysis. Make sure to protect the reagents from contaminated equipment and materials and decontaminate working surfaces before setting up the experiment.
- To prevent the presence of air bubbles in the array, also consider using the ***remove bubble wizard*** (3500 Genetic Analyzer) or ***Refresh PDS*** (SeqStudio™ Genetic Analyzer). The wizard ***Fill the array with fresh polymer*** (3500 Genetic Analyzer) or ***Fill array*** (SeqStudio™ Genetic Analyzer) helps to renew the gel in the capillaries and can minimize artefact formation from e. g. crystallized polymer or if capillaries are dye-

contaminanted from previous runs. An empty Hi-Di™-Formamide run helps also cleaning the capillaries.

- The robustness analysis of the Matrix Standard BT5 multi showed that the kit is robust against medium deviations from the described protocol (+/- 20 %). Higher deviations can result in failure of matrix generation, due to high background detected or insufficient number of dye spectra detected. To minimize any deviations, we recommend using calibrated pipettes, precise pipetting and thorough mixing.

Performance evaluation

The Matrix Standard BT5 multi serves as an accessory to the IVD products Mentype® AMLplex^{QS} PCR Amplification Kit, Mentype® Chimera® PCR Amplification Kit and Mentype® DIPscreen PCR Amplification Kit, functioning as a multicomponent matrix crucial for the spectral alignment of various dyes. As it is only intended to enable the medical devices fulfill their intended purpose(s), not all performance evaluation parameters are applicable to it. Its primary role lies in facilitating accurate spectral alignment for the forementioned IVD products.

Additionally, the Genetic Analyzer software's algorithm assesses the correct arrangement of the described fluorophores by examining the dye order, in addition to two primary parameters crucial for the determination of spectral alignment success: the spectral Q-value and the Condition Number. The spectral quality value indicates the confidence in separating individual dye emission signals from the overall fluorescence signal, reflecting the consistency between the final matrix and the source data. The Condition Number indicates the degree of overlap among dye peaks in the fluorescence emission spectra of the dye set. As peak overlap increases, the condition number rises accordingly. Subsequently, the software determines whether a capillary "passes" or "fails" the spectral alignment criteria based on these two predefined values.

During the performance evaluation, the consistency of the Matrix Standard BT5 multi functionality was assessed by verifying the successful spectral alignment across various capillary lots and instruments (as detailed in chapter Instruments and software).

The robustness analysis showed, that the Matrix Standard BT5 multi is not sensitive to pipetting deviations of +/- 20% or +/- 10% from the described protocol. Higher pipetting deviations were not tested can result in failure of matrix generation, due to high background detected or insufficient number of dye spectra detected. Furthermore, the product's stability was examined through multiple freeze-thaw cycles, its claimed shelf life, and shipping conditions. The product demonstrated stability for up to 6x freeze-thaw cycles, and its claimed shelf life was validated, aligning with the expiry date indicated on the label. Lastly, shipping stability was confirmed to meet the claimed shelf life after transportation.

Quality control

All kit components undergo an intensive quality assurance process at BIOTYPE GmbH. Quality of the test kit is permanently monitored to ensure unrestricted usability. Please contact support@biotype.de 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

Limitations of use

- The procedures in these instructions for use must be followed as described. Any deviations from protocol may result in kit failure or influence subsequent analyses.
- Use of this product is limited to laboratory professional users specially instructed and trained in PCR techniques and capillary gel electrophoresis.
- Appropriate storage and processing procedures are required for the optimal performance of this test.
- The product must not be frozen and thawn more than 6 times.
- The kit has only been validated for use with 3500 Genetic Analyzers and SeqStudio™ Genetic Analyzers. Please refer to chapter Instruments and software.
- Good laboratory practice is required to ensure the performance of the kit.

Ordering information

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

Product	Packaging size	Order number
Matrix Standard BT5 multi	25 reactions	45-15100-0025
	50 reactions	45-15100-0050
Mentype® Chimera® PCR Amplification Kit	25 reactions	45-12200-0025
	100 reactions	45-12200-0100
	400 reactions	45-12200-0400
Mentype® DIPscreen PCR Amplification Kit	25 reactions	45-12300-0025
	100 reactions	45-12300-0100
Mentype® AMLplex^{QS} PCR Amplification Kit	25 reactions	45-12100-0025
	100 reactions	45-12100-0100
	400 reactions	45-12100-0400
Mentype® AMLplus Mutation Analysis Kit	25 reactions	BT00005
	100 reactions	BT00006
ChimerisMonitor IVD	Demo license 1-year license 3-year license	46-14800-0000

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Other trademarks: Applied Biosystems® (Applied Biosystems LLC group); POP-4™, POP-7™, POP-1™ (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 Matrix Standard BT5 multi is a CE-marked accessory for diagnostic purposes according to the European In Vitro Diagnostic Regulation (EU) 2017/746.

The Product is not licensed with Health Canada and not FDA cleared or approved. Not available in all countries.

Explanation of symbols

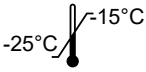
Manufacturer

Batch code

Contains sufficient for <N> tests

Consult electronic instructions for use

Use-by date



Temperature limit

Catalogue number

In vitro diagnostic medical device

Keep away from sunlight

Keep dry

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