Mentype® DIPscreen

PCR Amplification Kit

Instructions for Use (IFU)

IVD CE 0483 For in vitro diagnostic use

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BIOTYPE

Notice of Change

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

Document code	Changes	Date
DISIFU02v1en	Initial version	21.03.2025

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

For this or for any further questions, please contact us:

at +49 351 8838 400 or

support@biotype.de

Content

Intended Purpose

The Mentype® DIPscreen PCR Amplification Kit is a manual assay intended to be used with genomic DNA extracted from peripheral venous whole blood samples collected from adult leukemia patients that have undergone an allogenic hematopoietic stem cell transplantation (allo-HSCT).

The Mentype® DIPscreen PCR Amplification Kit detects 33 deletion insertion polymorphism (DIP) and amelogenin in one multiplex PCR reaction. Prior to the HSCT, these polymorphisms are used for the qualitative screening of the genotypes of patient and donor and to identify patient-specific DIP-alleles. These informative alleles are analyzed to perform a semi-quantitative chimerism monitoring after the allo-HSCT.

The Mentype® DIPscreen PCR Amplification Kit is intended for professional users trained in molecular-genetic techniques, multiplex PCR and the handling of Genetic Analyzers of Thermo Fisher Scientific (Applied Biosystems division).

Scientific Background

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a treatment option to cure patients with non-malignant and malignant hematological diseases, such as leukemia. Chimerism analysis is used to determine the mixture of donor and recipient hematopoietic cells in allo-HSCT recipients to detect early signs of graft rejection. Human peripheral venous blood is used for genotyping and monitoring. According to the CLSI Guidelines (MM05-A2 2nd Edition) anticoagulants like EDTA and citrate are recommended for blood collection. Depending on the success of transplantation different forms of hematopoietic chimerism (complete, mixed or loss) can develop. Different approaches are used for chimerism analysis, including fluorescence in situ hybridization (FISH), restriction fragment length polymorphism (RFLP), blood count analysis and PCR-based methods. The analysis of insertion-deletion polymorphisms (INDEL or DIPs) allows a semi-quantiative chimerism analysis with multiplex PCR approaches as well as quantitative analysis with allele-specific application on qPCR or dPCR platforms. Therefore, such DIP-assays are prerequisite for a sensitive chimerism monitoring. To detect early signs of graft rejection,

chimerism analysis should be done in regular intervals and shortly after the allo-HSCT.

Product Description

Mentype® DIPscreen PCR Amplification Kit is a multiplex polymerase chain reaction (PCR) amplification kit developed for chimerism monitoring. For donor-recipient discrimination, biallelic insertion-deletion polymorphisms (INDEL or DIPs) with a very high rate of heterozygosity and a balanced allelic distribution over 19 chromosomes (see Table 1) are simultaneously amplified in a single PCR reaction. One primer for each locus is labelled with the fluorophore 6-FAM™, BTG, or BTY. PCR products are then analyzed by capillary gel electrophoresis.

Table 1 Locus-specific information of Mentype® DIPscreen PCR Amplification Kit, HLD = Human Locus DIP, -DIP = Deletion, +DIP = Insertion

DIP Locus	Chromosomal position	DIP Locus
FAM Panel		
AM X	Xp22.1-22.3	
AM Y	Yp11.2	
HLD106	16q13	-/AATGCGT
HLD70	6q16.1	-/AGCA
HLD84	8q24.12	-/CTTTC
HLD103	12q23.1	-/GCTTATAA
HLD104	13q32.1	-/ACTC
HLD116	18p11.22	-/AGGTGTCGAACAACATGATAC
HLD112	17p12	-/TTGTA
HLD307	Xp11.23	-/TCAACCAA
HLD310	2p22.3	-/GTCTGGTT
HLD110	16q22.1	-/TCCCTG
HLD133	3p22.1	-/CAACCTGGATT
HLD79	7q31.2	-/AATCT
HLD105	14q24.3	-/ATAGACAA
HLD140	3q23	-/GGTAGTATGGGCCT
HLD163	12q24.31	-/AACTACGGCACGCCC
BTG Panel		
HLD91	11q14.1	-/GATA
HLD23	18p11.32	-/CTTTAA
HLD88	9q22.33	-/CCACAAAGA
HLD101	15q26.1	-/GTAG
HLD67	5q33.3	-/CTACTGAC
HLD301	17q21.32	-/CAGGGGCTC
HLD53	3q22.1	-/ATGT

DIP Locus	Chromosomal position	DIP Locus
HLD97	13q13.1	-/AGAGAAAGCTGAAG
HLD152	16p13.2	-/TGGTCAAAGGCA
HLD128	1q31.3	-/ATTAAATA
HLD134	5q11.2	-/ATGATGGTTCTTCAGA
HLD305	20q11.22	-/CAAGGTCCCACCACACTCGCGTGGGA
BTY Panel		
HLD48	2q11.2	-/GACTT
HLD114	17p13.2	-/TCCTATTCTACTCTGAAT
HLD304	9q34.3	-/GAGCTGCTCAAGAGAGAGG
HLD131	7q36.2	-/TTGGGCTTATT
HLD38	1q32.2	-/TAGTT
HLD82	7q21.3	-/ ACCTCCTACTCCTTGGTCTATTCCTG GTCACATGTACT

The assay was validated by chimerism analysis of over 200 HLA-matched related donor-recipient-pairs and its suitability was confirmed in a comparative clinical evaluation study.

The detection limit for the qualitative analysis is 125 pg genomic DNA.

The input range for mixed chimerism detection under standard conditions is 1.0 – 2.0 ng gDNA. The optimum gDNA input is 2 ng under standard conditions.

A sensitivity of 1.4 % LoD_{95} (Limit of Detection) can be achieved using 2 ng of gDNA as input, for the analysis of informative markers. To reach the aforementioned sensitivity, it is recommended to use as many informative markers as possible.

Materials provided

Kit content

Table 2 Mentype® DIPscreen PCR Amplification Kit content

Reagent	Cap color		Volume per packaging size	
			25 reactions	100 reactions
Nuclease-Free Water	Light blue		1.5 mL	2 x 1.5 mL
Reaction Mix A	Purple		125 µL	500 µL
Mentype® DIPscreen Primer Mix	Red		125 µL	500 µL
Multi Taq 2 DNA Polymerase	White		15 µL	60 µL
Control DNA XY82 (2 ng/µL)	White		10 µL	10 µL
DNA Size Standard 550 (BTO)	Orange		13 µL	50 µL
Mentype® DIPscreen Allelic Ladder	Green		25 µL	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 testings **without** taking into account the number of required controls or the required excess for pipetting.

We recommend using the following size for the corresponding throughput:

- < 8 samples per PCR run: 25 reaction packaging size
- ≥ 8 samples per PCR run: 100 reaction packaging size

Description of Components

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

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

Mentype® DIPscreen 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.

Control DNA XY82 (2 ng/µL): genomic DNA isolated from EDTA-blood of a single-source human male. The DNA is to be used as qualitative, external positive control for the Mentype® DIPscreen Amplification Kit.

NOTE

The Control DNA XY82 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 - 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® DIPscreen Allelic Ladder: mixture of artificial PCR products representing all alleles detected by the assay, 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 a 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 at -25 °C to -15 °C, protected from light. Especially the Mentype® DIPscreen Primer Mix, DNA Size Standard 550 (BTO) and Mentype® DIPscreen Allelic Ladder must be stored protected from light.

In order to prevent contamination, we recommend that pre-amplification components (DNA samples, the Control DNA XY82) and the post-amplification components (DNA Size Standard 550 (BTO) and Mentype® DIPscreen Allelic Ladder) are stored and used separately from PCR reagents (Nuclease-Free Water, Multi Taq 2 DNA Polymerase, Reaction Mix A and Mentype® DIPscreen Primer Mix).

The kit will expire according to the information on the kit box label or 12 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 materials used for PCR should 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 manufacturer's instructions and recommendations.

Reagents, kits and consumables

Table 3 Reagents required, but not provided

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
ChimerisMonitor IVD	BIOTYPE GmbH	46-14800-0000
QIAamp® DSP DNA Blood Mini Kit (IVD)	Qiagen	61104
NucleoSpin® DX Blood (IVD)	Macherey-Nagel	740899.50
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
3500 Genetic Analyzer 8-Capillary Array 36 cm	Thermo Fisher Scientific	4404683
3500 Genetic Analyzer 8-Capillary Array 50 cm	Thermo Fisher Scientific	4404685

Instruments and software

The Mentype® DIPscreen 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, cat. no. N805-0200, Thermo Fisher Scientific)
- Mastercycler nexus gradient (cat. no.: 6331000017, Eppendorf AG)
- Biometra Tadvanced (cat. no.: 846-2-070-214, Analytik Jena)

Only one of the above-listed instruments is required to perform the test.

The application of other instruments than the previously 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 - 5 °C/s

The Mentype® DIPscreen 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

The Mentype® DIPscreen PCR Amplification Kit was validated to be used with the following softwares. Only one of the below-listed softwares is required to analyze and evaluate data. A manual evaluation of the fsa-files or any results given with the software for data collection without one of the two software options described for data analysis and evaluation is not validated.

- ChimerisMonitor IVD (BIOTYPE GmbH)
- GeneMapper™ ID-X software, version 1.6 (Thermo Fisher Scientifc), using product specific:

- Analysis Method: DIPscreenIVD_Analysis4_v1x or DIPscreenIVD_Analysis7_v1x
- Bin: DIPscreenIVD_Bins4_v1x or DIPscreenIVD_Bins7_v1x
- Panel: DIPscreenIVD_Panel4_v1x or DIPscreenIVD_Panel7_v1x
- Size Standard: BTO_60-550_v1x

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 specimens have been validated with the Mentype® DIPscreen PCR Amplification Kit :

- Genomic DNA isolated from human peripheral venous whole blood (EDTA, citrate, heparin) for monitoring and genotyping.

The isolated gDNA shall be stored undiluted at -25 °C to -15 °C.

NOTE

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

Warnings and Precautions

- Read the 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 our homepage (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 and good laboratory practice.
- Do not use a kit that has passed its expiration date.
- Discard samples and assay waste according to your local safety regulations.
- All instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

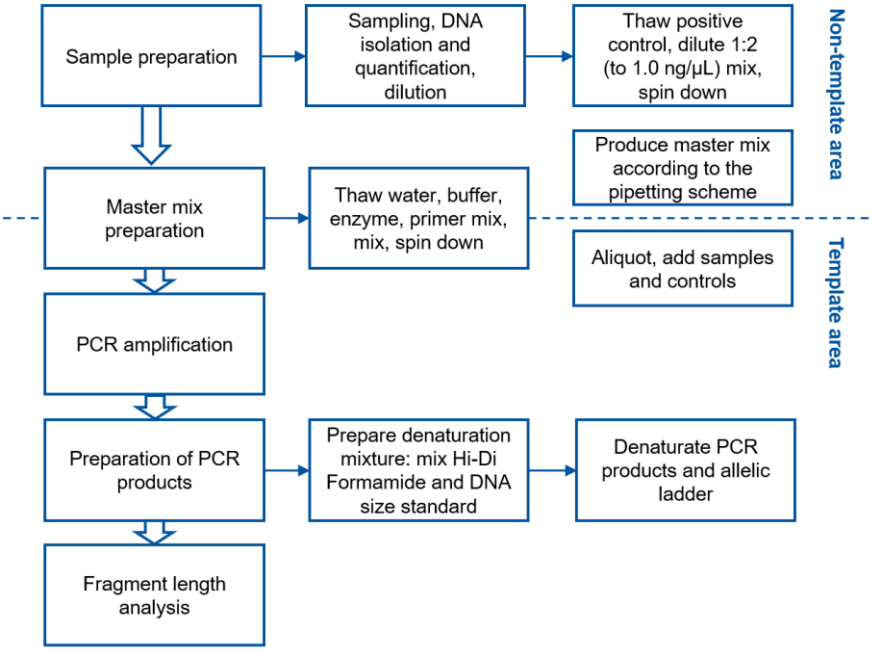
Notice to the user

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

Take at least a 200 µL sample of peripheral venous whole blood for the following procedure. The handling of the raw sample material (peripheral venous whole blood) should follow the recommendation of the Clinical and Laboratory Standards Institute (CLSI) guideline MM05–A2 (2nd edition), where they state that whole blood can be stored at room temperature (22 °C to 25 °C) for up to 24 hours, or at 2 °C to 6 °C for 72 hours or more. Additionally, it is recommended that the anticoagulants used for whole blood collection are EDTA, citrate or heparin.

NOTE

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 incomplete profiles.

DNA extraction

Perform the DNA extraction and purification from peripheral venous whole blood samples according to the manufacturer's instruction. The following kits have been verified as part of the performance evaluation of the product:

- QIAamp® DSP DNA Blood Mini Kit
- Macherey-Nagel NucleoSpin® Dx Blood, CE certified Mini Kit for DNA from Blood

NOTE

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

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

DNA quantification and dilution

Quantify the DNA concentration by UV/VIS spectroscopy at 260 nm using the NanoDrop spectrophotometer or by fluorescence spectroscopy using the Qubit™ Fluorometer.

When using spectrophotometry, use the elution buffer from the DNA extraction kit to measure the blank. The A_{260}/A_{280} ratio should be in the range of 1.7 – 1.9, whereas the A_{260}/A_{230} ratio should be in the range of 1.8 – 2.3.

For the fluorometric quantification of the DNA, the Qubit™ Fluorometer with either the Qubit 1x dsDNA HS Assay-Kit or the Qubit dsDNA BR Kit can be used.

For use with Mentype® DIPscreen PCR Amplification Kit, dilute the DNA samples to an optimal concentration of 2.0 ng/µL. Prepare the dilution freshly before usage. Use nuclease-free water as diluent.

NOTE

The total input range for the kit is 0.125 – 2.0 ng DNA per reaction, the **optimum input is 2 ng DNA** per reaction under standard conditions for highest sensitivity. An input below 0.125 ng might lead to incomplete profiles, a higher input might lead to pull-up peaks.

DNA storage

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

Control preparation

Positive control PC

Thaw the Control DNA XY82, homogenize it by vortexing followed by brief centrifugation.

Dilute the Control DNA XY82 1:2 from 2.0 ng/µL to 1.0 ng/µL using the Nuclease-Free Water.

Homogenize the diluted PC by brief vortexing. After this, briefly centrifuge the diluted PC (approx. 10 s). Do not store the diluted positive control.

NOTE

Always apply a fresh dilution of the Control DNA XY82.

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 the following components from the Mentype® DIPscreen PCR Amplification Kit for the master mix setup:

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

All frozen components should be thawed at room temperature (22 °C to 28 °C, ca. 30 min, protected from light) and homogenized by inverting the tubes or gentle vortexing. After this, briefly centrifuge the reagents (approx. 10 s). To uphold the principles of good laboratory practice, it is advisable to keep the Multi Taq 2 DNA Polymerase in a cooled environment as long as possible (e. g. cooling rack) prior to the master mix setup.

NOTE

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

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

NOTE

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

Table 4 PCR master mix reaction setup, * The volume depends on the DNA concentration. If a higher volume of DNA template is used make sure to adjust the volume of Nuclease-Free Water. The total reaction (rxn) volume must be 25.0 µL at all times.

	Volume		
Component	**1 rxn**	**5 rxn**	**10 rxn**
Nuclease-Free Water*	13.4 µL	67.5 µL	134.0 µL
Reaction Mix A	5.0 µL	25.0 µL	50.0 µL
Mentype® DIPscreen Primer Mix	5.0 µL	25.0 µL	50.0 µL
Multi Taq 2 DNA Polymerase	0.6 µL	2.4 µL	6.0 µL
DNA template or control sample	1.0 µL*	5 x 1.0 µL*	10 x 1.0 µL*
Total volume	**25.0 µL**	**125.0 µL**	**250.0 µL**

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

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

Application of DNA 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.

Sample: add 1.0 µL of the prepared, diluted gDNA samples (2.0 ng/µL).

PC: add 1.0 µL of the prepared, 1:2 diluted Control DNA XY82 (1.0 ng/µL) instead of a sample.

NOTE

First, prepare the NTC to avoid contaminations of the control. Prepare the PC 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, make sure to set the ramping to 4 - 5 °C/s. Perform a “hot start” PCR in order to activate the polymerase and to prevent the formation of non-specific amplification products.

Table 5 PCR protocol

Temperature	Time	
94 °C	4 min	
94 °C	30 s	
60 °C	120 s	28 cycles
72 °C	75 s	
68 °C	60 min	
10 °C	∞	hold

NOTE

If thermal cyclers with adjustable heating and cooling steps are used, **ramping shall be adjusted to 4 - 5 °C/s** in order to provide an optimal signal 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.

NOTE

Very small amounts of DNA may result in statistical dropouts and imbalances of the peaks. Increasing the number of PCR cycles raises the risk of cross-contamination caused by minimal amounts of impurities. Furthermore, unspecific amplification products could appear.

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 up to 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® DIPscreen 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 in the Genetic Analyzer).

Add either 1.0 µL PCR product or 1.0 µL Mentype® DIPscreen 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 evaluation.

NOTE

The capillaries of the gel electrophoresis device should 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® DIPscreen 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 Series 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: DIPscreenIVD_Instrument436.xml POP-7™, 50 cm capillary array: DIPscreenIVD_Instrument750.xml
Size Standard Protocol	BTO_60-550_SizeStandard.xml
Sizecalling Protocol	BTO_60-550_Sizecalling.xml
Assay	POP-4™, 36 cm capillary array: DIPscreenIVD_Assay436.xml POP-7™, 50 cm capillary array: DIPscreenIVD_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

	Injection 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

Differing from the values given in Table 8, the run time can be adjusted according to the capillary array length used, but it is mandatory to analyze all fragments (60 – 550 bp) of the DNA Size Standard 550 (BTO).

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

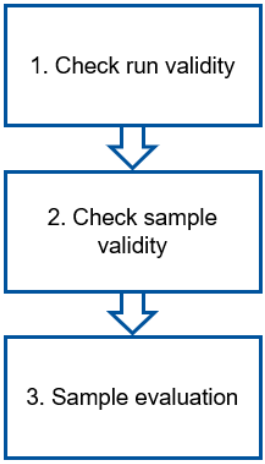
60, **80**, **90**, **100**, **120**, **140**, **160**, **180**, **200**, **220**, **240**, **250**, **260**, **280**, **300**, **320**, **340**, **360**, **380**, **400**, **425**, **450**, **475**, **500**, **525**, and **550** bp.

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

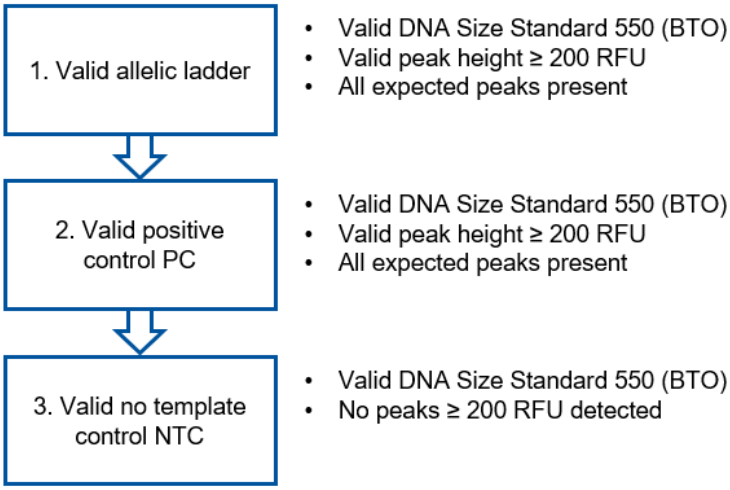


- See chapter **General run evaluation**
- See chapter **Sample analysis**
- See chapter **Qualitative analysis – identification of informative loci**
- See chapter **Semi-quantitative analysis – chimerism analysis**

NOTE

The data analysis must be performed either with the ChimerisMonitor IVD Software or the GeneMapper™ ID-X Software (Thermo Fisher Scientific). A manual evaluation of the fsa-files or any results given with the software for data collection without one of the two software options described for data analysis and evaluation is not validated.

Workflow run validation



NOTE

The measuring range 50 - 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 loci, 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
- All fragments are present with peak heights above the cut-off **≥ 50 RFU**
- Coefficient of determination **R^2 > 0.995**.

- The fragments do not continuously decrease in peak height with increasing fragment length.

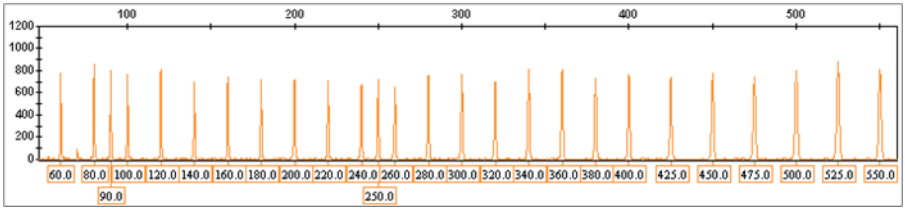


Figure 1 Electropherogram of the DNA Size Standard 550 (BTO), fragments with lengths in bp

Mentype® DIPscreen Allelic Ladder

After ensuring a valid size standard, check that all peaks available in the allelic ladder are present with peak heights above the cut-off **≥ 200 RFU**.

NOTE

The Mentype® DIPscreen Allelic Ladder includes fragments for each detectable alleles. Please compare the alleles with appendix Table 20 and Figure 5.

Control DNA XY82 (PC)

After ensuring a valid size standard, make sure that a complete DNA-profile of all specific peaks for the PC is present with peak heights of **≥ 200 RFU** (see Table 9). The Control DNA XY82 is a qualitative PCR control to ensure master mix performance in general.

The Control DNA XY82 (see appendix Figure 6), which is part of the test kit, represents the following alleles:

Table 9 Genotype of Control DNA XY82, - = Deletion, + = Insertion

Locus	Control DNA XY82	Locus	Control DNA XY82
FAM Panel (blue channel)		**BTG Panel (green channel)**	
Amelogenin	X/Y	HLD91	+/+
HLD106	+/+	HLD23	-/+
HLD70	-/+	HLD88	+/+
HLD84	+/+	HLD101	-/+
HLD103	-/+	HLD67	-/+
HLD104	-/+	HLD301	-/+
HLD116	-/+	HLD53	+/+
HLD112	-/+	HLD97	-/+
HLD307	+/+	HLD152	-/+
HLD310	-/+	HLD128	-/+
HLD110	-/+	HLD134	+/+
HLD133	-/+	HLD305	+/+
HLD79	+/+	**BTY Panel (Yellow channel)**	
HLD105	-/-	HLD48	-/-
HLD140	-/+	HLD114	-/-
HLD163	-/+	HLD304	-/+
		HLD131	+/+
		HLD38	+/+
		HLD82	+/+

No template control NTC

After ensuring a valid size standard, check that no peaks above the cut-off **≥ 200 RFU** are detected within the bin range of the NTC (see appendix Figure 6).

NOTE

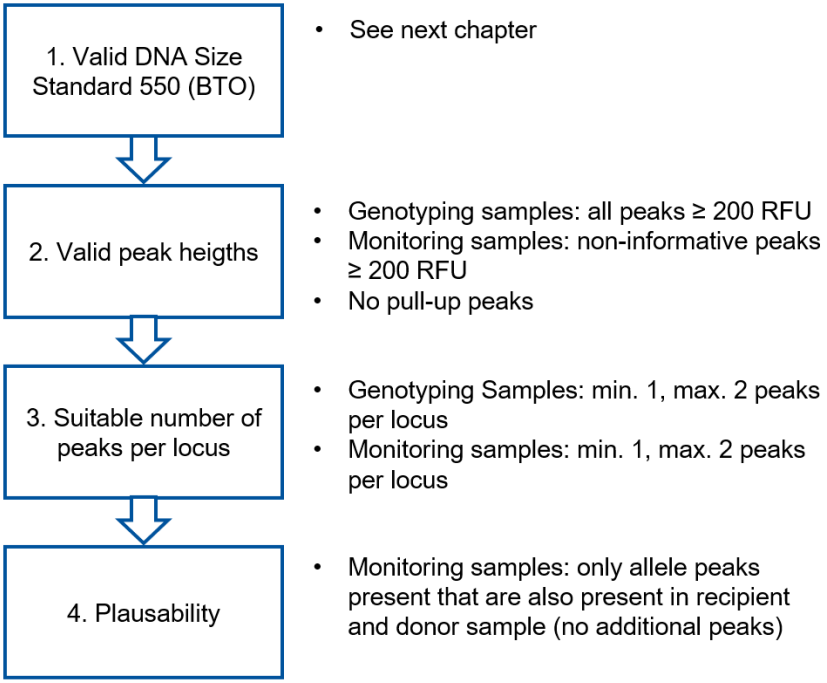
Using ChimerisMonitor IVD or GeneMapper™ ID-X together with the provided template files for the Analysis Method, peaks < 200 RFU are automatically not assigned with the allele name, supporting you to easily evaluate the results.

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



Using the software ChimerisMonitor IVD, the described validation steps for the run and the samples are implemented automatically.

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

Qualitative analysis – identification of informative loci

In the following section, the identification and differentiation of recipient specific loci is explained. Therefore, donor specific loci are defined as non-informative. The identification of informative loci is performed using data from recipient and donor before the transplantation. For examples, see Table 21 - Table 22 in the appendix.

Using ChimerisMonitor IVD, the identification of informative loci is supported by the software.

Informative Loci: One allele in the recipient sample cannot be detected in the donor sample.

Non-informative loci: Loci where the recipient specific peak(s) overlap with the donor specific peak(s), or donor-specific loci.

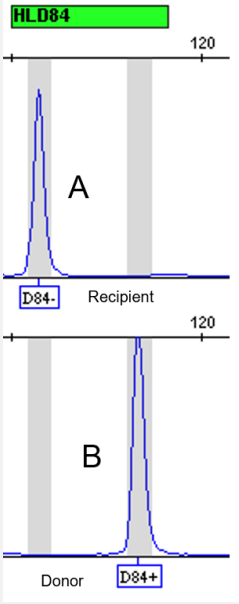
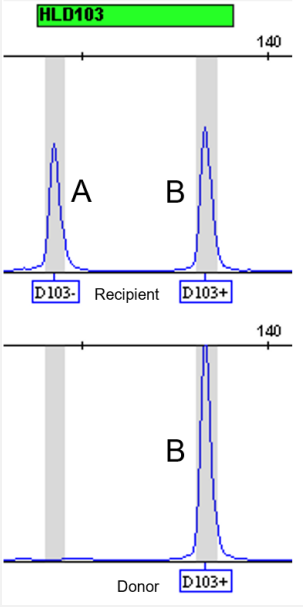
Semi-quantitative analysis – chimerism analysis

The semi-quantitative chimerism analysis is performed as described in the literature, e. g. Clark et al. (2015) [1] or Nollet et al. (2001) [2]. The formulas for the quantification depend on the allelic constellation in the locus and are shown in the following Table 10 (adapted from Clark et al.). The chimerism value is calculated for each previously selected informative locus. Then, the mean of all locus-specific chimerism values is calculated.

NOTE

The evaluation of each SD_{Marker} is recommended to identify outliers. The SD_{Marker} for low mixed chimerism samples (< 5 % MC) should not exceed 4 %. For medium to high mixed chimerism (> 5 % MC) the SD_{Marker} should not exceed 10 %. If the SD_{Marker} exceeds the limits, it is recommended to re-analyze the MC values of the chosen markers for outliers.

Table 10 Semi-quantitative chimerism analysis (adapted from Clark et al. [1])

Scenario 1	Scenario 2
no shared alleles: recipient is homozygous, donor is homozygous, no peaks are shared	**one shared allele (homozygous):** recipient is heterozygous, donor is homozygous, one peak is shared
	
Recipient ratio: $\% \text{ chimerism} = \frac{A}{A+B} \times 100\,\%$	**Recipient ratio:** $\% \text{ chimerism} = \frac{A}{(\frac{(B-A)}{2}) + A} \times 100\%$

Data analysis with ChimerisMonitor IVD

ChimerisMonitor IVD is an advanced software for an automated data analysis, run evaluation and chimerism calculation. The integrated ***Patient Management*** system allows to monitor chimerism kinetics in high resolution reports, but also in graphs and tabular visualization.

For general instructions on the sample analysis refer to the ChimerisMonitor IVD software instructions for use.

All required analysis templates are included in the ***Test Kit Management*** system of ChimerisMonitor. Those contain analysis methods as well as linked Bin and Panel templates. The software performs a general, integrated run evaluation during the batch import according to the chapter Workflow run validation. See Table 11 for a general description of the ChimerisMonitor data analysis workflow.

NOTE

The data analyis must be performed either with the ChimerisMonitor IVD Software or the GeneMapper™ ID-X Software (Thermo Fisher Scientific). A manual evaluation of the fsa-files or any results given with the software for data collection without one of the two software options described for data analysis and evaluation is not validated.

Table 11 Chimerism analysis workflow with ChimerisMonitor

No.	Icon	Working step
1	Sample Import	
		***Create new patient***. A database of all created patients is represented in the Patient Management ***Batch Import***: - Select the test kit ***Biotype Mentype DIPscreen*** All thresholds for the correct run and sample evaluation are linked to the respective analysis method. - Import a run containing fsa-files of the allelic ladder, positive control, no template control, and the samples. - Select sample types manually (essential for correct peak assignment and chimerism calculation)

No.	Icon	Working step
		- General run and sample evaluation is carried out by the software
		Open the ***Batch Import View***
		***Assign Sample***: Select a sample and assign it to the patient
2	Check controls – ChimerisMonitor performs an integrated quality assessment as well as a run and sample evaluation	
		Check the ***Allelic Ladder Electropherogram*** and ***Size Calling Regression*** Possible quality warnings are displayed: - Within tab ***Evaluation of Allelic Ladders*** during the Batch Import - Within tab ***FSA Import Warnings*** in the Patient Editor
		Check the ***Positive Control Electropherogram*** and ***Size Calling Regression*** The ***Evaluation of Positive and No Template Controls*** during the Batch Import displays possible quality warnings.
		Check the ***No Template Control Electropherogram*** and ***Size Calling Regression*** The ***Evaluation of Positive and No Template Controls*** during the Batch Import displays possible quality warnings
3	Sample evaluation	
		Check the ***Sample Electropherogram*** A correct peak assignment is essential for an accurate definition of informative markers and a robust chimerism calculation. The ***Sample Quality*** Check during the Batch Import displays possible quality warnings
		Check the Sample's Size Calling Regression The ***Sample Quality*** Check during the Batch Import displays possible quality warnings
4	Definition of informative markers	
		***Create a new transplantation***: predefined markers can be selected for patient monitoring
5	Chimerism Analysis	
		***Calculate Chimerism***: See preselected markers for chimerism analysis and carry out chimerism calculation (single marker chimerism, total chimerism and standard deviation)
6	Report	

No.	Icon	Working step
		***Create Report***: Single values and chimerism kinetics are displayed over time (table and graph, file format pdf or csv)
7		Build a database-driven system for ***Patient Management***

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® DIPscreen PCR Amplification Kit template files from BIOTYPE GmbH. The BIOTYPE template files (see Table 12) are available on our homepage (https://www.biotype.de/en/template-files) for download, or upon request via support@biotype.de. The chimerism analysis workflow using the GeneMapper™ ID-X software is shown in Table 13.

Table 12 BIOTYPE GmbH templates for GeneMapper™ ID-X Software, templates specific for # POP-4™ or § POP-7 ™ application

Template	Template name	
Panels*	DIPscreenIVD_Panel4_v1x# DIPscreenIVD_Panel7_v1x§	or higher versions
Bin Sets*	DIPscreenIVD_Bins4_v1x# DIPscreenIVD_Bins7_v1x§	or higher versions
Size Standard*	BTO_60-550_v1x	or higher versions
Analysis Method*	DIPscreenIVD_Analysis4_v1x# DIPscreenIVD_Analysis7_v1x§	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 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 13 Chimerism analysis workflow with GeneMapper™ ID-X

No.	Icon	Working step
1		Software Preparation
		***Panel Manager*** Import the provided template files for Panel, Bins
		***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 GmbH template DIPscreenIVD_Analysis_v1x
Panel	Select the previously imported BIOTYPE GmbH template DIPscreenIVD_Panel_v1x
Size Standard	Select the previously imported BIOTYPE GmbH template BTO_60-550_v1x

No.	Icon	Working step
4		Check controls
		Check control validity (Allelic Ladder, Positive Control, No Template 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
		If peaks are not assigned although sufficient heights are reached, a manual assignment is possible. Please check all peak assignments for plausibility.
6		Definition of informative markers
		Compare genotypes of Recipient and Donor, then identify informative markers manually (See Appendix, Examples for the qualitative evaluation of loci)
7		Chimerism Analysis
		Export the ***Sizing Table*** and calculate chimerism values according to Table 10 - see Chapter Semi-quantitative analysis – chimerism analysis

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

The post-PCR analysis and automatic allele assignment with suitable analysis software ensures a precise and reliable discrimination of alleles.

An automated calculation of the donor/recipient DNA ratio, as well as standard deviations and detection limits, can be obtained directly from the raw data of a fragment size analysis.

If results that are obtained with Mentype® DIPscreen PCR Amplification Kit should be harmonized with results from cytological analyses, make sure that cytological analyses were performed with at least 200 leucocytes.

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 can appear at positions of specific peaks in other colour panels, typically with lower signal intensities. For regular observation please consider repeating the matrix generation and check for DNA overload.

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 final extension step of the PCR protocol at 68 °C for 60 min. The peak height of the artefact correlates with the amount of DNA. 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 can 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 and, maybe, using more than one allelic ladder sample per run.

Artefacts like small dye blobs may also occur in samples, and even 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.

Pipetting deviations

The robustness analysis of the Mentype® DIPscreen PCR Amplification Kit showed that the kit is robust against minor deviations from described protocol (+/- 10 % deviation). A medium deviation (+/- 20 %) from the described experimental protocol may be more critical for the analysis of mixed chimerism (particularly at low chimerism percentages). It is crucial to pay special attention to the Reaction Mix A PCR Buffer. A deviation of - 20 % in this buffer can result in a significant reduction in signal height or even undetectable markers. To minimize any deviations, we recommend using calibrated pipettes, precise pipetting and thorough mixing.

Outliers

The evaluation of the standard deviation within markers (SD_{Marker}) is recommended to identify outliers. If the SD_{Marker} for low mixed chimerism samples (< 5 % MC) exceeds 4 % and for medium to high mixed chimerism samples (> 5 % MC) 10 %, please consider re-analyzing MC values without this specific outlier.

Influence of polymers

The Mentype® DIPscreen PCR Amplification Kit was validated and certified for the analysis on POP-4™ and POP-7™ polymer.

When using polymer POP-7™, dye blobs may occur in the front part of the electropherograms. In particular, dye blobs have been observed until the size of 110 bp in the blue channel and might affect the HLD106 locus. Check the

electropherograms for such effects before performing the chimerism analysis.

The use of other polymers (e. g. POP-6™) might influence the run behaviour of specific PCR products. Furthermore, background noise might increase through the different behaviour of free fluorescent dyes.

Performance Evaluation

Analytical specificity

We tested the automatic allele calling with the allelic ladder and the concordance of the allele assignment compared to the pre-typing of the test DNAs by means of other methods (other PCR kits) using the Genemapper™ ID-X software.

We evaluated eighty-one pre-characterized DNA samples, analyzed using the Mentype® DIPscreen PCR Amplification Kit. Complete profiles with peak heights ≥ 200 RFU were detected. After determination of the test-specific device settings, the correct genotype was assigned to all DNA samples for all DIP systems and the amelogenin marker. Additionally, we evaluated the primer specificity of the Mentype® DIPscreen PCR Amplification Kit. No unspecific mispriming PCR products were observed, thus, ensuring the primer specificity of the Mentype® DIPscreen PCR Amplification Kit.

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), EP37 (1st edition). For the Mentype® DIPscreen 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 were tested and in case interference was observed, further concentrations were tested to determine the concentration at which no interference is detectable.

Results showed an interfering effect of the endogenous interferent blood tested at 2.57E- 03 % v/v in PCR reaction on the measurement of the full

profile of single genotype DNA. Moreover, deviations from the untreated group were observed for the mixed chimerism samples measured at 2 %, 5 %, and 30 % MC. The results were outside the accuracy limits and thus showed interference on the PCR reaction. However, blood contamination can be visually detected in the PCR reaction or in the isolated DNA suspension by an orange color shift. Trained laboratory professional users should observe if a color change is present and repeat the DNA isolation to avoid any interference.

For the exogenous interferents EDTA, Sodium Citrate, Ethanol, Acetylsalicyllic Acid, Metoclopramide, and Cyclosporine showed no interference effect at their respective C_{max}, where the measurement of the full profile of single genotype DNA XY1726 could be achieved, and deviations from the untreated group observed for the mixed chimerism samples measured at 2 %, 5 %, and 30 % MC were within the accuracy limits.

As for the exogenous interferents Heparin, Proteinase K, and Methotrexate interference was observed at the C_{max} recommended by CLSI EP37 (1st edition). Further dilutions were tested until no interference was detectable. For Heparin 2.36E- 02 mg/dL, for Proteinase K 3.22E- 06 % v/v, and for Methotrexate 13.6 mg/dL 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 expect the workflow of the isolation kits to eliminate the tested interfering substances originating from anticoagulants and DNA isolation reagents. As for the chemotherapeutic agent Methotrexate, it showed no interference at the tested concentration when compared to its reference blank.

Table 14 tested concentrations of endogenous and exogenous interferents.

Type of interferent	Category	Interferent	Non-interfering concentration
Endogenous	Whole blood components	Whole blood	not tested
Exogenous	Anticoagulants	EDTA	0.099 mg/dL
		Sodium, Citrate	8.23E- 05 % v/v in PCR
		Heparin	2.36E- 02 mg/dL
	DNA isolation agents	Proteinase K	3.22E- 06 % v/v in PCR reaction
		Ethanol	2.70E- 03 % v/v in PCR Reaction
	Analgesics and antipyretics	Aspirin (Acetylsalicylic acid)	3 mg/dL
	Antiemetic agent	Metoclopramide	0.225 mg/dL
	Chemotherapeutic agent	Methotrexate	13.6 mg/dL
	Immunosuppressant agent	Cyclosporine	0.18 mg/dL

Analytical sensitivity

Single-genotype human gDNA is measurable in the range of 0.125 ng to 2.00 ng. In this range, full profiles of all DIP markers and amelogenin can be measured without defects. We recommend 1.00 ng as optimal input amount of single-genotpye DNA for acceptable full-profile analysis while reducing the material consumption. For mixed chimerism detection with optimal sensitivity, we tested different input amounts. To avoid restricted sensitivity for low input amounts, we defined 2.00 ng of DNA as the optimal input amount for mixed chimerism detection.

The linearity was evaluated based on the CLSI - EP06Ed2 guideline (2nd edition). Linearity was tested using six different mixed chimerism samples. Each mixed chimerism sample was diluted to cover the recipient chimerism from 1 % to 90 % (1 %, 2 %, 5 %, 10 %, 30 %, 50 %, 70 %, 90 %). The acceptable deviation from linearity (ADL) was defined with 4 % for samples up to 10 % recipient chimerism to allow only minor deviations in this range. For recipient chimerism > 10 %, the ADL was set to 10 % to allow for medium deviations from linearity. The expected and predicted values were below the ADL for all samples, confirming the linearity for the tested chimerism measuring range.

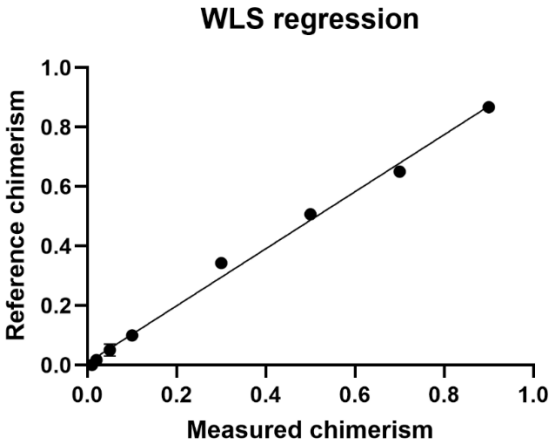


Figure 2 Example for weighted regression of different chimerism concentrations for one chimerism mixture. The regression showed a coefficient of determination of 0.994 confirming a high degree of linearity between the measured and the expected values over the complete test range of 1 % to 90 % recipient chimerism.

The Limit of Blank (LoB) was tested on 31 single-genotype DNAs on three different days with two different kit lots. The LoB for mixed chimerism was calculated using the non-parametric approach of CLSI - EP17-A2 (α = 0.05, 2nd edition). Only informative markers were analyzed. The result confirmed a LOB of 0.49 %.

Using 2.00 ng DNA input amount, we tested three different mixed chimerism samples with concentrations of 0.50 % to 3.00 %. All samples were tested with two different kit lots. Using the Probit-Analysis (CLSI - EP17-A2,

2nd edition) with a confidence level of 95 % the resulting Limit of Detection (LoD) for Lot 1 was 1.36 % and 1.39 % for Lot 3. The resulting reportable LoD is 1.39 % mixed chimerism.

Table 15 LoD for Mentype® DIPscreen PCR Amplification Kit using informative markers

Lot	LoD (% mixed chimerism)	N_{total}
Lot 1	1.36 %	252
Lot 3	1.39 %	252

The precision goal for the limit of quantification was defined according to CLSI - EP17-A2 (2nd edition). For the detection of chimerism at the LoD we defined the precision goal using the Westgard model (TE = IbiasI + 1.96 s; (Westgard et al. 1974 [3])) and the bias and precision of the assay. The resulting pooled standard deviation with 0.45 % was below the TE-based precision goal of 1.77 %, confirming an acceptable accuracy at the LoD.

Accuracy

The imprecision and bias can vary between different contents of chimerism. We concluded to divide the mixed chimerism (MC) range into three intervals also reported by Pettersson et al. (2021) [4] to establish adapted acceptance criteria for each interval. The intervals are:

- Low MC with < 5 % content of second genotype
- Medium MC with 5 - 20 % content of second genotype
- High MC with > 20 % content of second genotype

The analysis of accuracy was based on the CLSI – EP21Ed2E guideline (2nd edition). The bias measured in comparison with reference material and the imprecision (reproducibility) measured in a multisite study was used to calculate a sigma value based on the Sigma metric (Westgard et al. 2018 [3]). The Total Error acceptable was established based on the bias estimates of the linearity study and reported imprecision for STR-based PCR assays for chimerism analysis (Pettersson et al. 2021 [4]). The resulting sigma

values showed good accuracy for low and medium MC interval and excellent accuracy for high MC interval.

Table 16 Sigma-values for mixed chimerism

Sample	Sigma value
2 % mixed chimerism	3.08
5 % mixed chimerism	3.05
30 % mixed chimerism	4.99

Trueness

Bias evaluation was based on reference material from three different ring trial providers (3 different External Quality Control programs). The bias was calculated for the difference between Mentype® DIPscreen PCR Amplification Kit result and the reference value:

$$\Delta MC = Reference\ MC - Mentype\ DIPscreen\ MC$$

The bias calculation is based on CLSI - EP09Ed3cE (3rd edition) guideline and used the average of ∆MC values as normal distribution was proven (Shapiro-Wilk normality test, α = 0.05). According to the reference values, the samples were divided into the three sub-intervals for MC as described above (chapter Accuracy). The resulting bias ranged from -0.68 to 0.74.

Table 17 Bias (average of ∆MC) for low, medium and high MC

	low MC (< 5 %)	medium MC (5-20 %)	high MC (> 20 %)
Bias (average of ∆MC)	0.54	0.74	-0.68

Beside bias evaluation we compared the Mentype® DIPscreen PCR Amplification Kit with a reference method. The reference method of the PCR-based Mentype® Chimera® PCR Amplification Kit analyses short tandem repeats (STR) polymorphism to separate between two individuals (genotypes). Both methods were measured with 97 patient samples representing different mixed chimerism content. The resulting coefficient of

determination of 0.9963 confirmed a high correlation between the two methods.

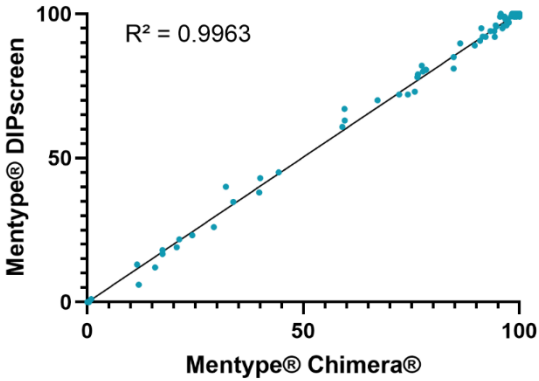


Figure 3 Reference Method comparison of Mentype® DIPscreen PCR Amplification Kit and Mentype® Chimera® PCR Amplification Kit. The linear regression to the right shows an acceptable correlation with a coefficient of determination of 0.9963.

Precision

We evaluated the assay repeatability and reproducibility based on the ISO 5725-2:2022-05 and the CLSI - EP05 (3rd edition). Mixed chimerism samples covering the chimerism measuring range (2 %, 5 %, 30 %, 70 %) were evaluted in an 5 x 5 x 3 (day x replicate x site) multisite study. The resulting repeatability for mixed chimerism ranged between 0.60 % to 1.68 % standard deviation (SD) of mixed chimerism and reproducibility ranged between 0.63 % to 1.75 % SD of mixed chimerism.

Assay Cutoff

We evaluated the assay cutoff for the Mentype® DIPscreen PCR Amplification Kit based on the calculation of the allelic frequency of the different amplified markers. Samples of 81 individuals were analyzed with the Mentype® DIPscreen PCR Amplification Kit. The resulting allelic frequencies were used to calculate specific forensic discrimination parameters also relevant for chimerism analysis such as the polymorphism

information content (PIC), expected heterozygosity (HET) and power of discrimination (PD) which demonstrates the ability of a genetic marker or set of markers to distinguish between different individuals within a population. The PIC and HET values showcase the highly polymorphic INDELs and a high rate of heterozygosity for the Mentype® DIPscreen PCR Amplification Kit. The PD for all DIP markers ranges from 0.5146 to 0.6581 which technically allows the discrimination of two individuals based on at least one marker, but the PD increases when using more markers in combination.

Table 18 Discrimination probability of all markers included in the Mentype® DIPscreen PCR Amplification Kit. PIC, PD and HET were calculated for each marker based on analysis of 81 Individuals.

Marker	PIC	HET	PD
AM	0.3384	0.4341	0.4664
HLD106	0.3066	0.3804	0.5301
HLD70	0.3744	0.5019	0.6572
HLD84	0.3695	0.4921	0.6328
HLD103	0.3703	0.4938	0.6389
HLD104	0.3651	0.4835	0.6389
HLD116	0.3736	0.5003	0.5322
HLD112	0.3725	0.4982	0.5877
HLD307	0.3736	0.5003	0.6054
HLD310	0.3559	0.4660	0.6152
HLD110	0.3685	0.4901	0.6261
HLD133	0.3703	0.4938	0.6462
HLD79	0.3174	0.3981	0.5603
HLD105	0.3736	0.5003	0.6191
HLD140	0.3623	0.4783	0.5972
HLD163	0.3703	0.4938	0.6060
HLD91	0.3712	0.4954	0.5274
HLD23	0.3559	0.4660	0.5850
HLD88	0.3719	0.4969	0.5597
HLD101	0.3740	0.5012	0.6261
HLD67	0.3384	0.4341	0.5834
HLD301	0.3736	0.5003	0.6054
HLD53	0.3593	0.4724	0.6216

Marker	PIC	HET	PD
HLD97	0.3736	0.5003	0.6411
HLD152	0.3329	0.4246	0.5146
HLD128	0.3750	0.5030	0.6554
HLD134	0.3384	0.4341	0.5752
HLD305	0.3685	0.4901	0.5530
HLD48	0.3695	0.4921	0.6109
HLD114	0.3593	0.4724	0.6353
HLD304	0.3750	0.5031	0.6520
HLD131	0.3725	0.4982	0.6581
HLD38	0.3384	0.4341	0.5834
HLD82	0.3103	0.3865	0.5487
MIN	0.3066	0.3804	0.4664
MAX	0.3750	0.5031	0.6581

In-use stability

All stability studies were planned conformant with ISO 23640:2015, and the CLSI EP25 guideline (2nd Edition). The following procedure was conducted for all stability studies: The Mentype® DIPscreen PCR Amplification Kit was tested at multiple timepoints over various durations. The analysis of chimerism samples was performed using ChimerisMonitor IVD, single-genotype DNA of XY1726 and XX1180 represented the recipient and donor respectively.

We analyzed single-genotype DNA XY1726 at a DNA input amount of 1 ng and mixed chimerism samples (MC) of XY1726:XX1180 (recipient:donor) at a DNA input amount of 1 ng representing the different MC intervals of 4 % and 30 % MC. For the master-mix stability, we tested the different MC intervals of 30 %, 5 % and 2 % MC with 1 ng DNA input for 30 % and 5 % MC and 2 ng DNA input for 2 % MC.

The final evaluation of the various conditions included the comparison between the means of the starting timepoint (t_0) and the different time points (t_n) and was calculated using the following equation:

$$abs.\Delta_n = |\bar{t}_0 - \bar{t}_n|$$

For the In-Use Stability study, three experiments were performed. One to test the stability after exposure to freeze and thaw cycles, one to test the stability after different handling of the master mix and the other to test the stability of the kits during simulated use after opening.

All tested samples in the master-mix stability experiment presented a full marker profile for the single-genotype DNA XY1726 and were below the total error-based accuracy and precision limits of the assay for the three MC samples tested: 30 %, 5 % and 2 % MC with their limits of 3.31 %, 3.62 % and 1.77 % respectively. Thus, the Mentype® DIPscreen PCR Amplification Kit remains unaffected by a different handling of the master mix.

For the freeze and thaw stability, the Mentype® DIPscreen PCR Amplification Kit shows no critical unacceptable deviations from T_0 and a full marker profile for the single-genotype DNA XY1726 and were below the total error-based accuracy limits of the assay for the MC samples tested: 30 % and 4 % with their limits 3.31 % and 2.85 %. Thus, the Mentype® DIPscreen PCR Amplification Kit is stable for up to 20 freeze and thaw cycles.

Based on the results for the simulated use after opening, the Mentype® DIPscreen PCR Amplification Kit is stable for use after its first opening up to 12 months, as well as stable for up to 20 freeze and thaw cycles.

Clinical performance data

Study design, ethics and regulatory aspects

The Mentype® DIPscreen PCR Amplification Kit and reference methods were tested on patient samples after allo-HSCT. The 10 patients included in this study were monitored after allo-HSCT and the donor content of the samples during the monitoring was analyzed 10 times after allo-HSCT. The aim of this study was to provide clinical evidence according to §§ 20 to 24 of the medical device act ‘Medizinproduktgesetz’ (MPG version from 7 August 2002 (BGBI. I S 3146). Using the cytogenetic reference method FISH, a concordance with the deletion insertion polymorphism-based PCR method of the device had to be proven. The confirmation of the responsible ethic commission was received on 14.03.2012.

Reference methods

As reference method, Fluorescence-In-Situ-Hybridization (FISH) was conducted using allosome-specific CE-IVD CEP® X SpectrumOrange™ / Y SpectrumGreen™ Direct Labeled Fluorescent DNA Probe Kit (Abbott GmbH & Co KG, Wiesbaden). To apply the reference method, only donor-recipient pairs of different gender were included in the study. Only cytogenetic results with cell counts > 200 were evaluated, according to manufacturers' recommendations.

DNA extraction and purification

DNA was isolated using the QIAamp® DNA Blood Mini Kit (Qiagen GmbH, Hilden, DE). The isolation was conducted according to manufacturers protocol.

Results

The high correlation between the Mentype® DIPscreen PCR Amplification Kit and the cytogenetic reference method with $R^2 = 0.9889$ confirms the applicability for chimerism monitoring after allo-HSCT.

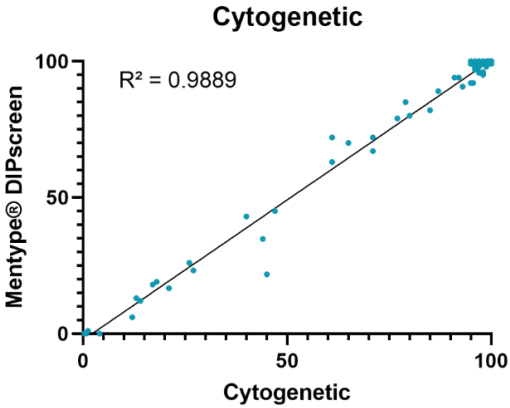


Figure 4 The correlation of cytogenetic and Mentype® DIPscreen PCR Amplification Kit results are shown in the graph. The linear regression sows an acceptable correlation with a coefficient of determination of 0.9889 (N = 87)

The resulting concordance of 94.25 % (5 % ΔMC accepted) with the reference method results confirm the reliability of the Mentype® DIPscreen PCR Amplification Kit for clinical data interpretation.

Diagnostic Evaluation

The clinical performance characteristics showed acceptable results. The results were evaluated concerning a LoD of PCR and cytogenetic of 1 % (Bader et al. 2023 [5]). The parameters for clinical performance evaluation according to Annex I, Sec. 9.1b of the IVDR were found to be not completely applicable for the Mentype® DIPscreen PCR Amplification Kit. The device monitors an analyte range rather than the presence of an analyte. Thus, an assessment of negative and positive status of the analyte is not completely applicable.

Table 19 Diagnostic characteristics

Diagnostic characteristics	Estimate	Lower Confidence Interval	Upper Confidence Interval
Diagnostic Sensitivity	96.0 %	90.6 %	100.0 %
Diagnostic Specificity	76.3 %	62.8 %	89.8 %
Diagnostic Accuracy	87.5 %	80.6 %	94.4 %
Positive predictive value	84.2 %	74.7 %	93.7 %
Negative predictive value	93.5 %	84.9 %	100.0 %
Prevalence	56.8 %	46.5 %	67.2 %

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 us if you have any questions regarding quality assurance.

Technical Assistance

For technical advice, please contact our Customer Support Team:

e-mail: support@biotype.de

phone: +49 (0)351 8838 400

References

[1] Clark J, Scott S, Jack A, Lee H, Mason J, Carter G, Pearce L, Jackson T, Clouston H, Sproul A, Keen L, Molloy K, Folarin N, Whitby L, Snowden J, Reilly J, Barnett D (2014) Monitoring of chimerism following allogeneic haematopoieticstem cell transplantation (HSCT): Technical recommendationsfor the use of Short Tandem Repeat (STR) based techniques,on behalf of the United Kingdom National External Quality Assessment Service for Leucocyte Immunophenotyping Chimerism Working Group. *British Journal of Haematology 168, 26–37.*

[2] Nollet F, Billiet D, Selleslag D, Criel A (2001) Standardisation of multiplex fluorescent short tandem repeat analysis for chimerism testing. *Bone Marrow Transplantation 28, 511-518.*

[3] Westgard S, Bayat H, Westgard JO (2018) Analytical Sigma metrics: A review of Six Sigma implementation tools for medical laboratories, Biochemia medica

[4] Pettersson L, Vezzi F, Vonlanthen S, Alwegren K, Hedrum A, Hauzenberger D (2021) Development and performance of a next generation sequencing (NGS) assay for monitoring of mixed chimerism, Clinica Chimica Acta

[5] Bader P, Bornhäuser M, Grigoleit G-U, Kröger N für die DAG-KBT, Deutsche Arbeitsgemeinschaft für Knochenmark- und Blutstammzelltransplantation e.V. Monitoring, Chimärismusanalysen und Bestimmung der minimalen Resterkrankung (MRD). Allogene Stammzelltransplantation. Onkopedia Leitlinien. Status March 2023 www.onkopedia.com.

Limitations of Use

- The procedures in this IFU 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 instructed and 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.
- The kit has only been validated for use with human peripheral venous whole blood samples run on the PCR instruments listed in chapter Instruments and software
- 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 using the kits described in chapter Reagents, kits and consumables for DNA extraction and purification.
- Good laboratory practice is required to ensure the performance of the kit.
- Results must be interpreted in consultation with clinicians who combine results of chimerism analyses with results of other therapy- or diagnostic relevant methods.
- Interpretation of results must account for the possibility of false negative and false positive results.
- The Mentype® DIPscreen PCR Amplification Kit cannot be used if donor and recipient are identical twins.
- Double-transplantations were not validated as part of the performance evaluation.
- Specimen containing degraded DNA may affect the ability to detect the INDEL/DIP and sex-specific loci

- Do not use expired or incorrectly stored components.
- Chimerism analysis was validated using patient-specific informative markers (for all chimerism ranges).
- Specimens from different ethnic groups may exhibit distinct genetic characteristics such as varying allelic frequencies, which could limit the power of discrimination or amplification. The allelic frequency of some DIP markers across multiple populations has been evaluated. The combined power of discrimination is high with values exceeding 0.99. This suggests a relatively low risk that a patient's test will face amplification difficulties. However, population data are currently available for Caucasian. The potential impact for other ethnic groups cannot be estimated yet.

Ordering information

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

Product	Packaging size	Order number
Mentype® DIPscreen PCR Amplification Kit	25 reactions 100 reactions	45-12300-0025 45-12300-0100
Matrix Standard BT5 multi	1 x 25 µL 2 x 25 µL	45-15100-0025 45-15100-0050
ChimerisMonitor IVD	demo license 1-year license 3-year license	46-14800-0000

NOTE

Individual components of the kits can not be ordered separately.

Trademarks and Disclaimers

Mentype® and Chimera® are a registered trademarks of BIOTYPE GmbH.

Other trademarks: ABI PRISM®, GeneMapper®, GeneAmp® and Applied Biosystems® (Applied Biosystems LLC group); QIAamp® (Qiagen); POP-4™ (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® DIPscreen PCR Amplification Kit is a CE-marked diagnostic kit 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.

The medical device is not available in all countries.

Explanation of Symbols

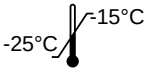
Manufacturer

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

Electropherograms of reference samples

On the following pages you can find examples of the electropherograms of the Mentype® DIPscreen Allelic Ladder (Figure 5), the Control DNA XY82 (PC, Figure 6) and a no template control (NTC, Figure 7).

All samples were amplified on a ProFlex PCR cycler and analyzed on an Applied Biosystems™ 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 templates and Analysis Method according to Table 12 were applied.

The electropherograms are zoomed to a fragment length of 70 – 420 bp (x-axis). The general range for fragment length analysis (x-axis) using the Mentype® DIPscreen PCR Amplification Kit is 50 bp to 550 bp. The scaling of the y-axis was performed individually according to the description below each figure (Figure 5, Figure 6, Figure 7).

Mentype® DIPscreen Allelic Ladder

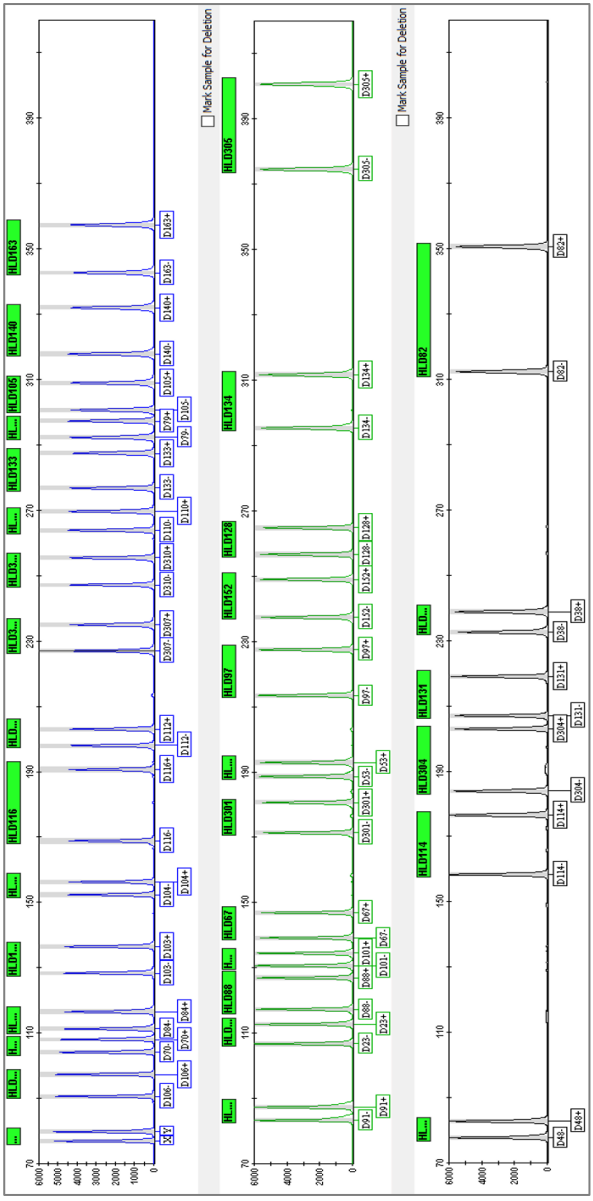


Figure 5 Mentype® DIPscreen Allelic Ladder
Zoom to 6,000 RFU (y-axis) and 70 – 420 bp (x-axis)

Control DNA XY82 (PC)

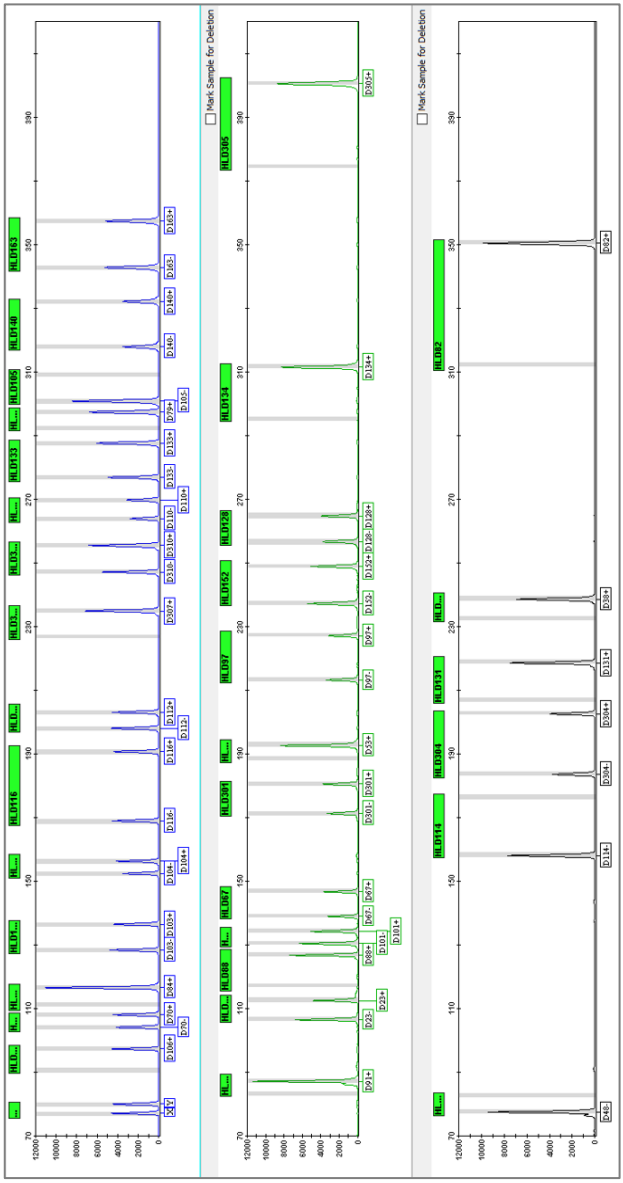


Figure 6 Control DNA XY82 (PC)
Zoom to 12,000 RFU (y-axis) and 70 – 420 bp (x-axis)

No template control (NTC)

Figure 7 No Template Control (NTC)
Zoom to 1,000 RFU (y-axis) and 70 – 420 bp (x-axis)

Lengths of fragments and alleles

Table 20 shows the fragment lengths of individual alleles that refer to the DNA Size Standard 550 (BTO). All analyses have been performed on an ABI PRISM® 310/3130 Genetic Analyzer with POP-4™ polymer. Different analysis instruments, DNA size standards or polymers may result in different fragment lengths. In addition, a visual alignment with the allelic ladder is recommended.

Table 20 Fragment lengths of the Mentype® DIPscreen Allelic Ladder analyzed on an ABI PRISM® 3130 Genetic Analyzer with POP-4™ polymer, * rounded to integer

Marker/ Blue channel	Size -DIP [bp]*	Size +DIP [bp]*	Marker/ Green channel	Size -DIP [bp]*	Size +DIP [bp]*
Amelogenin	77 (X)	80 (Y)	HLD91	84	88
HLD106	91	98	HLD23	107	113
HLD70	104	108	HLD88	118	128
HLD84	112	117	HLD101	131	135
HLD103	129	138	HLD67	140	148
HLD104	153	157	HLD301	172	182
HLD116	170	192	HLD53	190	194
HLD112	199	204	HLD97	214	228
HLD307	228	236	HLD152	239	250
HLD310	248	257	HLD128	258	266
HLD110	264	270	HLD134	296	312
HLD133	278	288	HLD305	375	401
HLD79	294	299	**Marker/ Green channel**	**Size -DIP [bp]***	**Size +DIP [bp]***
HLD105	302	310			
HLD140	318	333	HLD48	78	83
HLD163	344	358	HLD114	159	177
			HLD304	184	203
			HLD131	208	220
			HLD38	234	240
			HLD82	314	352

Examples for the qualitative evaluation of loci

Table 21 Examples for qualitative evaluation of informative loci

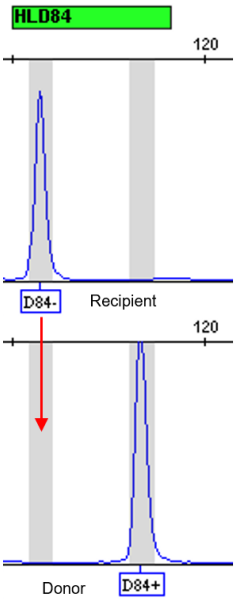
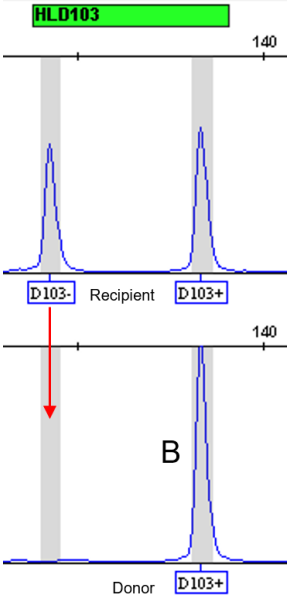
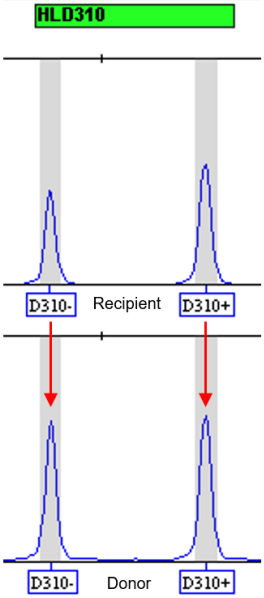
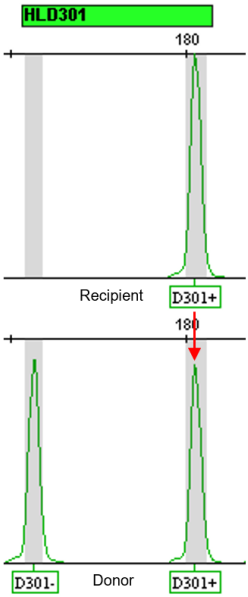
Informative Loci	Informative loci
	

Table 22 Example for qualitative evaluation of non-informative loci

Non-informative Loci	Non-informative loci
	

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