Mentype® DigitalQuant

Handbook

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

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

* xx - defines the locus-specific article number

BIOTYPE

Notice of Change

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

Document code	Changes	Date
DGQHB01v1en	Intial version	09.03.2022
DGQHB01v2en	Workflow inclusion QuantStudio Absolute Q exclusion of QuantStudio 3D (discontinuation)	13.02.2025
DGQHB01v3en	New article number for DIP Positive Control Workflow inclusion for QIAcuity Digital PCR System	11.04.2025

For any further questions, please contact us:

at +49 351 8838 400 or

support@biotype.de

Content

Product Description

The Mentype® DigitalQuant test kits were developed for the absolute quantification of DNA proportions from mixed samples.

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

The test kit should only be used by professional users trained in molecular biological techniques and in performing digital PCR in particular.

Summary and Explanation

The Mentype® DigitalQuant approach employs the highly sensitive digital PCR technology that allows for absolute quantification of DNA samples. Specific for digital PCR (dPCR) is the sample-partitioning into a plethora of compartments. Each compartment represents a separate reaction vessel containing nano-liters of the sample of interest. During thermal cycling each compartment functions as a separate PCR amplification chamber. Depending on how many copies of the target DNA molecules have been divided into the compartments (zero, one or more copies), a multitude of replicates is generated in each PCR run. Using Poisson statistics, the absolute number of starting copies can be determined very accurately. After thermal cycling, each compartment is automatically analyzed and determined as positive or negative fraction. Because the digital PCR uses end-point detection of the amplification product, calibration curves are not necessary.

Mentype® DigitalQuant assays were developed to quantitatively analyze identified DIP loci (deletion insertion polymorphisms). These assays represent a set of 29 different DIP alleles (INDELs) and are provided as duplex mixes either in combination with ß-globin as an active reference (REF) or with SRY as a Y-chromosome specific marker. Additionally, a combination of SRY/REF is provided as assay.

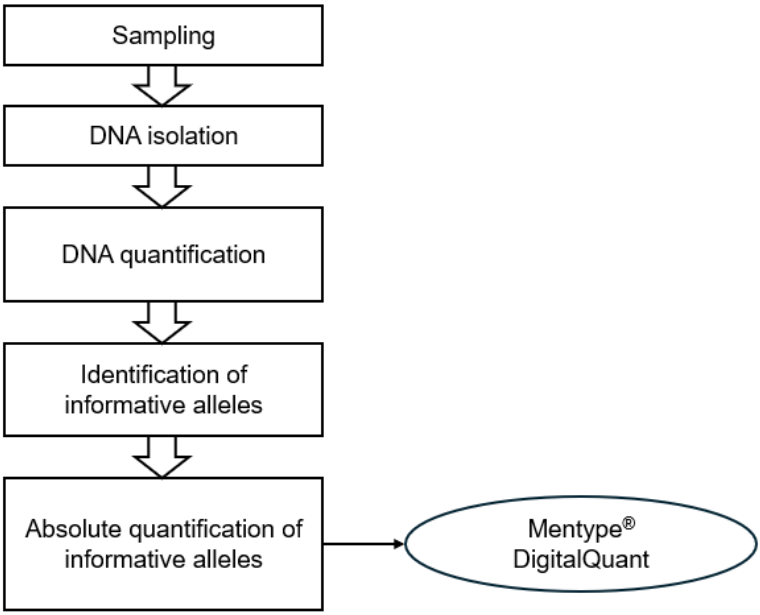

Figure 1 From sample to result with Mentype® DigitalQuant

Thereby, all specific DIP-Markers (see Table 18) are labeled with FAM. Accordingly, the active reference (REF) and the Y-chromosome specific markers (SRY) are detected in the HEX channel (for Absolute Q system the VIC channel is used). All assays were designed to run with the same parameters and allow parallel analysis of multiple DIP-markers in one run.

Materials provided

The following reagents for running up to 25 reactions with the Mentype® DigitalQuant kit are included:

Table 1 Content of the Mentype® DigitalQuant kits

Component	Cap color	Volume per kit	Storage
Nuclease-Free Water	Light blue	1 x 1.5 mL	−25 °C to −15 °C, protected from light
Mentype® DigitalQuant Primer Mix	Red	1 x 63 µL	

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.

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 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.

The expiry date of the kit is indicated on the kit box label. Do not exceed a number of 10 freeze-thaw cycles.

General material and devices required but not provided

General laboratory equipment

- Desktop centrifuge with a rotor for 2 mL reaction tubes
- Centrifuge with a rotor for microtiter plates
- Vortex mixer
- Calibrated adjustable pipettes with disposal aerosol tight filter tips
- Appropriate (depending on the device manufacturer) 200 µL 96-well reaction plates with suitable foil, PCR grade
- Suitable racks for 2 mL tubes
- Cooling rack suitable for 2 mL tubes
- Disposable powder-free gloves
- NanoDrop™ Spectrophotometer or Qubit Fluorometer
- PCR Workstation or Clean Bench

NOTE

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

General reagents and kits, independent from dPCR platform

Table 2 Reagents and kits required, but not provided

Reagent	Supplier	Order number
DIP Positive Control, 20 reactions	BIOTYPE GmbH	27-13201-0100
QIAamp DNA Blood Mini Kit, 50 Preps	Qiagen	51104
NucleoSpin Blood L Kit, 20 Preps	Macherey-Nagel	740954.20

Specimens and test samples

The following specimen has been verified with the Mentype® DigitalQuant kits:

- DNA extracted from peripheral venous blood samples
- DNA extracted from bone marrow

Warnings and Precautions

- Read this handbook carefully before using the product.
- Read the safety data sheets (SDS) for all BIOTYPE products, which are available via https://www.biotype.de/en/sicherheitsdatenblatter or on request. Please contact the respective manufacturers 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 personnel specially instructed and trained in PCR techniques.
- 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
    - Condition upon arrival (components are frozen).
- Do not use a kit that has passed its expiration date.
- Discard sample 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.
- The application of Mentype® DigitalQuant on other instruments than described needs to be verified under the responsibility of the user.

General Procedure

Sample preparation

Sampling and DNA extraction

This test is intended for the use of DNA extracted from peripheral blood samples as well as bone marrow. The use of other samples (e. g. sorted cells) must be independently validated by the user.

For DNA extraction, commercially available kits for isolating of genomic DNA should be used. The following kits are recommended for the DNA extraction:

- QIAamp DNA Blood Mini Kit (Qiagen)
- NucleoSpin Blood L Kit (Macherey-Nagel)

The DNA should be quantified directly after the extraction with the NanoDrop spectrophotometer or the Qubit fluorometer. Concentrated DNA can be adjusted to the required concentration by dilution with 1 x TE buffer.

DNA storage

Store the DNA samples at -25 °C to -15 °C. Undiluted DNA samples can be stored for 4 weeks at 2 °C to 8 °C or at -25 °C to -15 °C for long-term storage.

Preparation of control samples

Positive Control (PC)

Thaw the DIP Positive Control (DPC, sold separately), homogenize it by gentle vortexing followed by briefly centrifuging.

Apply the undiluted DPC instead of a sample.

No Template Control (NTC)

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

Optional: Determination of allelic state

If the allele status of the informative loci is not known, it must be determined before calculating the DNA proportions. To do this, analyze the initial sample with the corresponding informative Mentype® DigitalQuant marker. The use of 10 - 25 ng of DNA in the reaction is recommended. After analysis of the initial sample, the allele status is calculated.

If the percentage ratio of concentration (copies/µL) in the FAM channel (AOI, alleles of interest, DIP loci or SRY) to the concentration (copies/µL) in the HEX or VIC (REF) channel is less than 65 %, the AOI is heterozygous.

If the ratio is greater than 65 %, it is a homozygous marker. The resulting calculation formulas for the quantification can be found in - .

$$\text{Ratio [\%]} = \frac{(100 * \text{conc(copies/µL) AOI})}{\text{conc(copies/µL) REF}}$$

Notes on quantification

In general, quantification using Mentype® DigitalQuant is calculated by the ratio of the concentration of the informative DIP locus (FAM, concentration (copies/µL)) to the concentration of REF or SRY (HEX or VIC, concentration (copies/µL)) within one duplex assay.

NOTE

For a statistically reliable and robust DNA analysis, the analysis of at least 2 and optimally 3 informative loci is recommended.

Procedure for droplet digital™ PCR (ddPCR™ by Bio-Rad)

Specific equipment required but not provided

Reagents and consumables

Table 3 Specific reagents, instruments and consumables for the droplet digital PCR (Bio-Rad)

Equipment	Supplier	Order number
2x ddPCR™ Supermix for Probes (No dUTP), 5 x 1 mL	Bio-Rad Laboratories	1863024
FastDigest EcoRI, 800 reactions	Thermo Fisher Scientific	FD0274
QX200™ Droplet Generator	Bio-Rad Laboratories	17005227
PX1 PCR Plate Sealer	Bio-Rad Laboratories	17005226
QX200™ Droplet Reader	Bio-Rad Laboratories	17005228
Droplet Generation Oil for Probes, 10 x 7 mL	Bio-Rad Laboratories	1863005
ddPCR™ Droplet Reader Oil, 2 x 1 L	Bio-Rad Laboratories	17005221
DG8™ Cartridge Holder, 1x	Bio-Rad Laboratories	1863051

Equipment	Supplier	Order number
DG8™ Cartridges for QX200™/QX100™ Droplet Generator, 24x	Bio-Rad Laboratories	17005222
DG8™ Gaskets for QX200™/QX100™ Droplet Generator, 24x	Bio-Rad Laboratories	17005223
Pierceable Foil Heat Seals, 100x	Bio-Rad Laboratories	17005225
ddPCR™ 96-Well Plates, semi-skirted, 25x	Bio-Rad Laboratories	17005224
ddPCR™ Buffer Control for Probes, 2 x 4,5 mL	Bio-Rad Laboratories	1863052
96-well PCR foil	Several	Varying

Instruments and software

The test kit was validated using the Bio-Rad QX100™ and QX200™ Droplet Digital™ PCR System and the following thermocycler:

- Applied Biosystem GeneAmp® PCR System 9700 Aluminium and GeneAmp® PCR System 9700 Silver
- Eppendorf Mastercycler ep-S und Mastercycler nexus
- Biometra T1
- Bio-Rad DNA Engine PTC-200

Experimental setup

PCR Master mix setup

Prepare the following components and thaw the reagents as required and homogenize them. The reagents should be then briefly centrifuged (approx. 5 s). Keep the enzyme on a cooling rack during usage.

- Nuclease-Free Water (light blue cap, included in the kit)
- Mentype® DigitalQuant Primer Mix (red cap, included in the kit)
- EcoRI enzyme
- 2x ddPCR™ Supermix for Probes (No dUTP)

NOTE

Please note the shelf life of the 2x ddPCR™ Supermix for Probes (No dUTP) after opening, that should be stored and used for a maximum of 2 weeks at 4 °C after thawing.

Prepare the PCR master mix according to Table 4. When calculating the required master mix volume, consider the number of positive and negative control reactions. Add one or two reactions to this number to compensate for pipetting errors.

Table 4 PCR master mix setup

Component	Volume per reaction
Nuclease-Free Water	2.5 µL
2x ddPCR™ Supermix for Probes (No dUTP)	10.0 µL
Mentype® DigitalQuant Primer Mix	2.0 µL
FastDigest EcoRI enzyme	0.5 µL
Volume master mix/well	**15.0 µL**
DNA template (10 ng/µL) or control samples	5.0 µL

NOTE

The detection limit and sensitivity of the Mentype® DigitalQuant analysis depend on the quality and amount of DNA used. The allele-specific primer mix is optimized for maximum specificity and sensitivity (0.1 %) to the use of 50 ng of purified total DNA.

Gently mix the PCR master mix without generating bubbles followed by brief centrifugation. Aliquot 15.0 µL of the PCR master mixes in suitable 200 µL PCR tubes and add the appropriate samples. Seal the tubes and mix gently. Centrifuge the tubes briefly.

Application of DNA templates and controls

Add 5.0 µL of the following sample types to the prepared tubes containing the PCR master mixes.

NTC: add 5.0 µL of Nuclease-Free Water instead of a sample.

DNA sample: add 5.0 µL of the prepared, diluted DNA samples (10 ng/µL).

PC: add 5.0 µL of undiluted DIP Positive Control (DPC) instead of a sample.

Restriction digestion

The BIOTYPE Mentype® Digital assays are specific for EcoRI restriction digestion.

Restriction digestion of the DNA template to be analyzed prior to droplet generation is recommended. The digestion can be carried out directly in the PCR reaction vessel and the final PCR reaction mix (see Table 4). Use max. 1 µL of FastDigest EcoRI enzyme to digest up to 1 µg of genomic DNA in a total volume of 20 µL. For the use of Non-FastDigest EcoRI enzyme 2 units per 20 µL reaction are recommended.

After preparing the PCR tubes with PCR master mix, sample DNA and controls, close them, mix thoroughly and centrifuge briefly.

Then incubate the mix in a thermocycler for 10 min at 37 °C for restriction digestion.

Droplet digital™ PCR – droplet generation

Place the DG8 cartridge into the cartridge holder. Pipette each sample (20 µL of the digested PCR mix) up and down for 3 times before transferring the sample to the sample-wells of the DG8 cartridge (also see the general guidelines from Bio-Rad for droplet generation).

Start with pipetting digested PCR mix. Transfer samples in the DG8 cartridge from left to right. All 8 sample wells in the DG8 cartridge must be filled either with sample or 1x Bio-Rad Buffer Control (not provided).

After transferring all samples, fill 70 µL of Droplet Generation (DG) Oil in the bottom-line wells. All 8 oil wells have to contain DG oil.

Hook the gasket over the cartridge holder by using the holes on both sides.

NOTE

When filling the DG8 Cartridge, always use the holder provided. The DG oil may only be distributed into the 8 wells of the DG8 cartridge once all 8 wells have been filled with sample.

Place the filled DG8 cartridge into the QX100/QX200 droplet generator and start the droplet generation.

After droplet generation, the top wells of the cartridge contain droplet-samples. Transfer 40 µL of the droplet-samples into a 96-well PCR plate. Use an 8-channel pipette to save time.

Proceed the same way with all samples.

NOTE

Upon droplet generation, handle samples gently (no vortex, no spin-down). Start PCR amplification immediately, but no more than 2 hours after the first droplet generation has been completed.

Seal the 96-well PCR plate with Pierceable Foil Heat Seal and place the plate into the PX1 PCR Plate Sealer. Also refer to the Bio-Rad instructions in the PX1 PCR Plate Sealer Manual.

PCR amplification

When heat sealing is completed, place the 96-well PCR plate into a thermal cycler and start the program according to Table 5. Use a heated lid and set to 105 °C. Set sample volume to 40 µL.

*ramp rate depends on the PCR cycler and the block material:

- For PCR cycler with aluminium block use a ramp rate of 2 °C/s.
- For PCR cycler with silver block use 1 °C/s;
- If you cannot determine the block-material use a ramp rate of 1 °C/s.

Table 5 PCR protocol for Mentype® DigitalQuant (application on QX100/ QX200)

Temperature	Time	Cycles	Ramping*
95 °C	10 min	1 x	2 °C/s
94 °C 62 °C	30 s 60 s	40 x	
98 °C	10 min	1 x	
4 °C	∞	1 x	1 °C/s

Droplet reading

After the thermal cycling is finished, place the PCR plate into the holder of the QX100/QX200 Droplet Reader.

NOTE

Do not vortex and/or spin down the plate after cycling! Handle it with care to avoid droplet deterioration.

Open the software Quanta™Soft from Bio-Rad. Create the plate layout for your experiment (see Figure 2). Open the editor (Applied Well Settings) by double-clicking on a well in the plate layout. Assign the sample name, the type of experiment, and determine which assay corresponds to which fluorescence channel. Then the sample names of the DNA samples to be analyzed can be assigned.

For Mentype® DigitalQuant, please define the settings according to Table 6.

Table 6 Settings to be determined for analyzing the Mentype® DigitalQuant Assays in the Quanta™Soft

Sample and experiment types	Settings
Sample	
Name	Give a name
Experiment	Absolute Quantification (ABS)
Supermix	ddPCR Supermix for Probes (no dUTP)

Sample and experiment types	Settings
Target 1	
Name	Marker name e. g. DP67
Type	e. g. Ch 1 Unknown
Target 2	
Name	REF or SRY
Type	e. g. Ch 2 Unknown

NOTE

All specific DIP-Markers (see Table 17) are labeled with FAM. The reference (REF or SRY) is labelled with HEX accordingly.

After definition of the experiment click ***Run***.

The droplet reader counts fluorescence-positive and negative droplets for an absolute quantification of target DNA. Each sample-containing droplet is individually processed and verified for both FAM and HEX fluorescence. Data from at least 10,000 accepted droplets must be used for the concentration calculations. This value can be found in the tab ***Table*** of the Quanta™Soft software, column ***AcceptedDroplets***, or in the ***Events*** view, labelled as total.

Data Analysis

General evaluation

Load the plate in the Setup window of the Quanta™Soft Software (Bio-Rad). Click ***Analyze*** to open and analyze the data. Review the data in the 2D Amplitude channel to verify whether the automated threshold and the cluster separation is correct (see Figure 2).

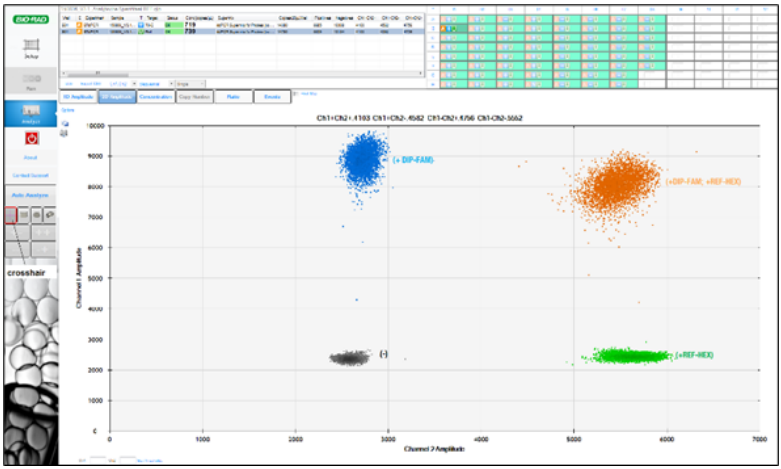

Figure 2 2D amplitude view (scatter plot) of the target fluorescence

All 4 clusters (grey, green, brown, blue) need to appear fully separated (see Figure 2). If clusters are not accurately separated or the automated threshold is not correct you have to draw corrections manually. Therefore, use the threshold adjustment tools (crosshair). The droplets are interpreted as follows:

- double negative (gray),
- FAM positive (blue),
- HEX positive (green) and
- double positive (orange - positive for FAM and HEX in the same droplet).

Manual correction was successful when the color of the cluster or individual droplets changes and the crosshairs are displayed in pink.

Then select the wells to analyze and click on ***Table***. Then open the ***Result Table*** to see the results.

NOTE

The detection limit for a successful experiment is five FAM-positive droplets. A result with less than five droplets is defined as negative, the FAM cluster was not recognized.

Minimum requirements for droplet data before calculation

Before the proportion of the mixed sample is calculated, the data should be checked for quality.

- At least 10,000 droplets should be detected and analyzed per sample.
- The detection limit for a successful experiment is five FAM-positive droplets. Here, the amplification in the FAM channel alone (blue cluster) and in the FAM and HEX-positive cluster (orange cluster) are present. A result with less than five droplets is defined as negative.

Procedure for QuantStudio™ Absolute Q™ (Thermo Fisher Scientific)

NOTE

Working with the Absolute Q system, the application of DP301-D + REF is not recommended due to reduced performance.

Specific equipment required but not provided

Reagents and consumables

Table 7 Specific reagents and consumables for the QuantStudio™ Absolute Q application

Equipment	Supplier	Order number
AbsoluteQ™ DNA Digital Master Mix (5x), 200 reactions	Thermo Fisher Scientific	A52490
QuantStudio™ Absolute Q™ MAP16 Plate Kit, 12 plates	Thermo Fisher Scientific	A52865

Instrument and software

The test kit was verified using the QuantStudio™ Absolute Q™ Digital PCR System (Thermo Fisher Scientific, A52864) and the following software :

- QuantStudio™ Absolute Q™ Digital PCR Software (version 6.2.0)

Experimental setup

PCR Master mix setup

Prepare the following components and thaw the reagents as required and homogenize them. The reagents should be then briefly centrifuged (approx. 5 s).

- Nuclease-Free Water (light blue cap, included in the kit)
- Mentype® DigitalQuant Primer Mix (red cap, included in the kit)
- AbsoluteQ™ DNA Digital Master Mix
- AbsoluteQ™ Isolation Buffer

Prepare the PCR master mix according to Table 8. When calculating the required master mix volume, consider the number of positive and negative control reactions. Add one or two reactions to this number to compensate for pipetting errors.

Table 8 PCR master mix setup

Component	Volume per reaction
Nuclease-Free Water	5.0 µL
AbsoluteQ™ DNA Digital Master Mix	2.0 µL
Mentype® DigitalQuant Primer Mix	1.0 µL
Volume master mix/well	**8.0 µL**
DNA template* (25 ng/µL) or control samples	2.0 µL

* for bad quality DNA samples the input volume can be increased to 7µL, when adapting the volume of Nuclease-Free Water accordingly.

NOTE

The detection limit and sensitivity of the Mentype® DigitalQuant analysis depend on the quality and amount of DNA used. The allele-specific primer mix is optimized for maximum specificity and sensitivity (up to 0.1 %) to the use of 50 ng of purified total DNA.

Gently mix the PCR master mix without generating bubbles followed by brief centrifugation. Aliquot 8.0 µL of the PCR master mixes in suitable 200 µL

PCR tubes and add the appropriate samples. Seal the tubes and mix gently. Centrifuge the tubes briefly.

Application of DNA templates and controls

Add 2.0 µL of the following sample types to the prepared tubes containing the PCR master mixes.

NTC: add 2.0 µL of Nuclease-Free Water instead of a sample.

DNA Sample: add 2.0 µL of the prepared, diluted DNA samples (25 ng/µL).

PC: add 2.0 µL of undiluted DIP Positive Control (DPC) instead of a sample.

NOTE

When using the Absolute Q platform, it is not necessary to perform a restriction digestion of the DNA template

Array loading

When handling the MAP16 plate, ensure that you only touch the outer frame to avoid damage and contamination. Perform all procedures on a flat, dust-free surface to maintain the integrity of the samples and the plate. To prevent cross-contamination, always use a new pipette tip for each well.

Load 9 µL of the PCR mix by holding the pipette at a 45° angle and dispensing the mix onto the bottom of the well. Press the pipette only to the first stop to avoid creating air bubbles. Add 15 µL of the Absolut Q Isolation Buffer in the same manner, holding the pipette at a 45° angle and pressing only to the first stop. Finally, place 5 gaskets onto the MAP plate, ensuring that the ends marked with "A" are aligned with the top side of the plate.

Place plate into the device.

Following these steps will help ensure accurate and contamination-free handling of the MAP16 plate and its components.

PCR amplification and run settings

Select the used columns of the MAP plate within the platemap in the software.

Perform the run using the amplification parameter from Table 9, select the detection of the FAM and HEX channel:

Table 9 PCR protocol for Mentype® DigitalQuant on Absolute Q

Temperature	Time	Cycles
96 °C	10 min	1 x
96 °C 62 °C	5 s 30 s	40 x
4 °C	∞	1 x

NOTE

Unused columns of the MAP plate can still be used in another run.

Data Analysis

General evaluation

Import the completed run in the ***Runs*** window of the Absolute Q™ Digital PCR Software (Thermo Fisher Scientific). Open the ***Analysis*** tab to see the results. Click on sample, select all channels and then choose the 2D plot view. Review the data in the 2D Amplitude channel to verify whether the automated threshold and the cluster separation is correct (see Figure 3).

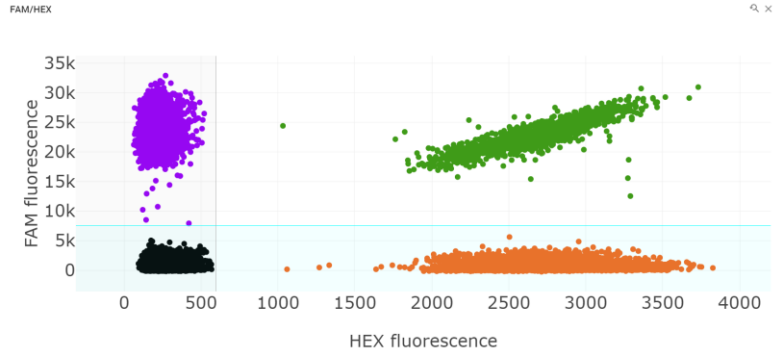

Figure 3 2D amplitude view (scatter plot) of the target fluorescence

All 4 clusters (black, green, orange, purple) need to appear fully separated (see Figure 3). If clusters are not accurately separated or the automated threshold is not correct, you have to draw corrections manually. Therefore, use the ***Edit Analysis*** option to adapt the thresholds. Please always refer to the positive control amplitudes when adapting thresholds manually. The events are interpreted as follows:

- double negative (black),
- FAM positive (purple),
- HEX positive (orange) and
- double positive (green - positive for FAM and HEX in the same cavity).

By saving the manual adaption the counts are calculated automatically by the software.

The analyzed data can be reviewed and downloaded from the ***Results*** tab.

NOTE

Due to differences in the signal amplitudes it is recommended to analyze a reference sample/ positive control on every plate helping to find the correct threshold, using the ***Group*** option in the Absolute Q™ Digital PCR Software.

Minimum requirements for data before calculation

Before the proportion of the mixed sample is calculated, the data should be checked for quality.

- Wells with less than 20,000 valid events will be excluded from evaluations by the software automatically.
- All clusters must be seperated according to the amplification profile of the positive control.

Procedure for QIAcuity Digital PCR System (Qiagen)

NOTE

Working with the QIAcuity Digital PCR system, the application of DP301-D + REF is not recommended due to reduced performance.

Specific equipment required but not provided

Reagents and consumables

Table 10 Specific reagents, instruments and consumables for the QIAcuity Digital PCR System (Qiagen)

Equipment	Supplier	Order number
QIAcuity Probe PCR Kit (5 mL)	Qiagen	250102
QIAcuity Nanoplate 26k 24-Well (10)	Qiagen	250001
QIAcuity Nanoplate Seals (11)	Qiagen	250099
QIAcuity Nanoplate Tray (2)	Qiagen	250098
QIAcuity Roller	Qiagen	911106

Instrument and software

The test kit was verified using the QIAcuity Digital PCR System (Qiagen GmbH, 911042) and the following software :

- QIAcuity Software Suite 2.5.0.1

Experimental setup

PCR Master mix setup

Prepare the following components and thaw the reagents as required and homogenize them. The reagents should be then briefly centrifuged (approx. 5 s).

- Nuclease-Free Water (light blue cap, included in the kit)
- Mentype® DigitalQuant Primer Mix (red cap, included in the kit)
- 4x QIAcuity Probe Mastermix

Prepare the PCR master mix according to Table 11. When calculating the required master mix volume, consider the number of positive and negative control reactions. Add one or two reactions to this number to compensate for pipetting errors.

Table 11 PCR master mix setup

Component	Volume per reaction
Nuclease-Free Water	21.0 µL
4x QIAcuity Probe Mastermix	10.0 µL
Mentype® DigitalQuant Primer Mix	4.0 µL
Volume master mix	**35.0 µL**
DNA template* (20 ng/µL) or control samples	5.0 µL

* When using higher DNA volumes, the volume of Nuclease-Free Water must be reduced accordingly. The total reaction volume must always be 40 µL.

NOTE

The detection limit and sensitivity of the Mentype® DigitalQuant analysis depend on the quality and amount of DNA used. The allele-specific primer mix is optimized for maximum specificity and sensitivity (up to 0.1 %) to the use of 100 ng of purified total DNA.

Gently mix the PCR master mix without generating bubbles followed by brief centrifugation.

Aliquot 35 µL of the PCR master mixes in suitable 200 µL PCR tubes or in a PCR plate.

Application of DNA templates and controls

Add 5.0 µL of the following sample types to the prepared tubes containing the PCR master mixes. Seal the tubes or the plate and mix gently. Centrifuge briefly.

NTC: add 5.0 µL of Nuclease-Free Water instead of a sample.

DNA Sample: add 5.0 µL of the prepared, diluted DNA samples (20 ng/µL).

PC: add 5.0 µL of undiluted DIP Positive Control (DPC) instead of a sample.

NOTE

When using the QIAcuity Platform, it is not necessary to perform a restriction digestion of the DNA template.

Array loading

Perform all procedures on a flat, dust-free surface to maintain the integrity of the samples and the plate. To prevent cross-contamination, always use a new pipette tip for each well.

Place the QIAcuity Nanoplate on the white Nanoplate Tray. Transfer the prepared 40 µL of the PCR mixture into the wells of the QIAcuity Nanoplate by holding the pipette at a 45° angle and dispensing the mix onto the bottom of the well, but without touching the bottom. Press the pipette only to the first stop to avoid creating air bubbles.

Seal the QIAcuity Nanoplate properly by using the blue Nanoplate Seals and the QIAcuity Roller.

Place the Nanoplate into the device without the Nanoplate Tray.

PCR amplification and run settings

Select the used columns of the QIAcuity Nanoplate and create a platemap in the QIAcuity Software Suite.

Perform the run using the amplification parameter from Table 12, select the detection of the FAM and HEX channel:

Table 12 PCR protocol for Mentype® DigitalQuant on QIAcuity

Temperature	Time	Cycles
95 °C	2 min	1 x
95 °C	15 s	40 x
61 °C	30 s	

Data Analysis

General evaluation

Open the completed run in the ***Plates*** window of the QIAcuity Software Suite (Qiagen GmbH). Open the ***Analyze*** tab to see the results under ***Absolute Quantification***. To set thresholds for the sample analysis, click on the plate position of the DIP Positive Control DPC. Select all targets and click ***Show results***. Clearly separate all clusters by choosing the automatic or a manual threshold (red lines, see Figure 4). Apply the threshold to all analyzed samples by selecting them in the plate view and inserting the values for both fluorescences.

Figure 4 2D amplitude view (scatter plot) of the target fluorescence

All 4 clusters (grey, yellow, light blue, dark blue) need to appear fully separated (see Figure 4) for all samples.

The events are interpreted as follows:

- double negative (grey),
- FAM positive (yellow),
- HEX positive (light blue) and
- double positive (dark blue - positive for FAM and HEX in the same cavity).

By saving the manual adaption the counts are calculated automatically by the software.

The analyzed data can be reviewed and downloaded from the ***List*** tab.

NOTE

Due to differences in the signal amplitudes it is recommended to analyze a reference sample/ positive control on every plate helping to find the correct threshold.

Minimum requirements for data before calculation

Before the proportion of the mixed sample is calculated, the data should be checked for quality.

- All clusters must be seperated according to the amplification profile of the positive control.
- If too many wells have been invalidated by the QIAcuity Software Suite, please consider reimaging. In order to achieve highest sensitivity it is advised to analyse 26k cavities.

Calculation of absolute DNA ratio

Calculate the ratio (concentration in copies/µL) of the DIP locus (AOI) to the reference (REF) or the SRY locus. Note whether it is a homozygous or heterozygous marker.

NOTE

Marker 307-I + REF is located on the X chromosome, thus in male-female mixed samples the marker has to be handled as heterozygous. The combination 307-I + SRY needs be calculated as shown in .

Table 13 Calculation for DIP+Ref combination: homozygous AOI

Sample	AOI	REF	Formula	Result
Post HSCT	Conc (copies/µL)	Conc (copies/µL)	= ((100 * AOI) / (REF)	
	338	644	= (100 * 338) / (644)	52.5 %

Table 14 Calculation for DIP+Ref combination: heterozygous AOI

Sample	AOI	REF	Formula	Result
Post HSCT	Conc (copies/µL)	Conc (copies/µL=	= ((100 * 2 * AOI) / (REF)	
	162	611	= (100 * 2 * 338) / (644)	53 %

Table 15 Calculation for DIP+SRY combination: homozygous AOI

Sample	AOI	SRY	Formula	Result
Post HSCT	Conc (copies/µL)	Conc (copies/µL)	= ((100 * AOI) / (AOI + 2 * SRY)	
	337	182	= (100 * 337) / (337 + 2 * 182)	48.1 %

Table 16 Calculation for DIP+SRY combination: heterozygous AOI

Sample	AOI	SRY	Formula	Result
Post HSCT	Conc (copies/µL)	Conc (copies/µL)	= ((100 * AOI) / (AOI + SRY)	
	553	506	= (100 * 553) / (553 + 506)	52.2 %

Characteristics and availability of Mentype® DigitalQuant assays

Table 17 Chromosomal location of the specific loci

Locus	Chromosomal Location	Locus	Chromosomal Location
67	5q33.3	133	3p22.1
70	6q16.1	134	5q11.2
88	9q22.33	140	3q23
97	13q13.1	152	16p13.2
101	15q26.1	163	12q24.31
104	13q32.1	301	17q21.32
105	14q24.3	304	9q34.3
106	16q13	307	Xp11.23
114	17p13.2	310	2p22.3
128	1q31.3	SRY	Yp11.2
131	7q36.2		

Table 18 Available allele-specific Mentype® DigitalQuant kits

Loci	Deletion (- Allel)	Insertion (+ Allel)	Allele specific duplex assay with Reference (REF)	Allele specific duplex assay with marker for Y chromosomal region (SRY)
67	67-D		DP67-D+REF	DP67-D+SRY
70	70-D		DP70-D+REF	DP70-D+SRY
		70-I	DP70-I+REF	DP70-I+SRY
88	88-D		DP88-D+REF	DP88-D+SRY

Loci	Deletion (- Allel)	Insertion (+ Allel)	Allele specific duplex assay with Reference (REF)	Allele specific duplex assay with marker for Y chromosomal region (SRY)
		88-I	DP88-I+REF	DP88-I+SRY
97		97-I	DP97-I+REF	DP97-I+SRY
101	101-D		DP101-D+REF	DP101-D+SRY
		101-I	DP101-I+REF	DP101-I+SRY
104	104-D		DP104-D+REF	DP104-D+SRY
		104-I	DP104-I+REF	DP104-I+SRY
105	105-D		DP105-D+REF	DP105-D+SRY
		105-I	DP105-I+REF	DP105-I+SRY
106		106-I	DP106-I+REF	DP106-I+SRY
114	114-D		DP114-D+REF	DP114-D+SRY
		114-I	DP114-I+REF	DP114-I+SRY
128	128-D		DP128-D+REF	DP128-D+SRY
131		131-I	DP131-I+REF	DP131-I+SRY
133		133-I	DP133-I+REF	DP133-I+SRY
134		134-I	DP134-I+REF	DP134-I+SRY
140		140-I	DP140-I+REF	DP140-I+SRY
152	152-D		DP152-D+REF	DP152-D+SRY
163	163-D		DP163-D+REF	DP163-D+SRY
		163-I	DP163-I+REF	DP163-I+SRY
301	301-D*		DP301-D+REF	DP301-D+SRY
		301-I	DP301-I+REF	DP301-I+SRY
304	304-D		DP304-D+REF	DP304-D+SRY
		304-I	DP304-I+REF	DP304-I+SRY
307		307-I	DP307-I+REF	DP307-I+SRY
310		310-I	DP310-I+REF	DP310-I+SRY
SRY		SRY	DPSRY+REF	

*only recommended for Bio-Rad analysis

Quality Control

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

Technical Assistance

For technical advice, please contact our Customer Support Team:

e-mail: support@biotype.de

phone: +49 (0)351 8838 400

References

Alizadeh M, Bernard M, Danic B, Dauriac C, Birebent B, Lapart C, Lamy T, Le Prise PY, Beauplet A, Bories D, Semana G, Quelvennec E. (2002) Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction. Blood 99, 4618-4625.

Vogelstein B, Kinzler KW (1999) Digital PCR. Proc Natl Acad Sci USA96:9236-9241.

George D, Czech J, John B, Yu M, Jennings LJ (2013) Detection and quantification of chimerism by droplet digital PCR. Chimerism, 4:102-108.

Manoj P (2014) Droplet digital PCR technology promises new applications and research areas. Mitochondrial DNA 2014, e-published ahead of print [doi: 10.3109/19401736.2014.913168].

Jim F. Huggett, Carole A. Foy, Vladimir Benes, Kerry Emslie,Jeremy A. Garson, Ross Haynes, Jan Hellemans, Mikael Kubista, Reinhold D. Mueller, Tania Nolan, Michael W. Pfaffl,Gregory L. Shipley, Jo Vandesompele, Carl T. Wittwer, and Stephen A. Bustin13 (2013) Guidelines for Minimum Information for Publication of Quantitative Digital PCR Experiments. Clinical Chemistry 2013.206375.

Limitations of Use

- The procedures in this handbook must be followed, as described. Any deviations may result in assay failure or cause erroneous results.
- Use of this product is limited to personnel specially instructed and trained in dPCR techniques.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The kit has only been verified using the kits and procedures described.
- Good laboratory practice is required to ensure the performance of the kit.
- Results must be interpreted by a trained professional user.
- Interpretation of results must account for the possibility of false negative and false positive results.
- Do not use expired or incorrectly stored components.
- The kit label indicates 25 reactions, which is based on the Bio-Rad platform, but the actual number of usable reactions may vary when using other digital PCR platforms.

Ordering information

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

Table 19 Ordering information for accessories: Mentype® DigitalScreen and DIP Positive Control

Product	Packaging size	Order number
Mentype® DigitalScreen	4 plates (4 x 2 DNA pairs)	45-64610-0004
DIP Positive Control	20 reactions	27-13201-0100

Table 20 Ordering information for allele specific Mentype® DigitalQuant assays, these assays are available to you anytime (warehousing)

Assay	25 reactions
DP67-D+REF	45-02011-0025
DP70-D+REF	45-02021-0025
DP70-I+REF	45-02031-0025
DP88-D+REF	45-02041-0025
DP88-I+REF	45-02051-0025
DP97-I+REF	45-02061-0025
DP101-D+REF	45-02071-0025
DP101-I+REF	45-02081-0025
DP104-D+REF	45-02091-0025
DP104-I+REF	45-02101-0025
DP105-D+REF	45-02111-0025
DP105-I+REF	45-02121-0025
DP106-I+REF	45-02131-0025
DP114-D+REF	45-02141-0025
DP114-I+REF	45-02151-0025
DP128-D+REF	45-02161-0025
DP131-I+REF	45-02171-0025
DP133-I+REF	45-02181-0025
DP134-I+REF	45-02191-0025
DP140-I+REF	45-02201-0025
DP152-D+REF	45-02211-0025
DP163-D+REF	45-02221-0025
DP163-I+REF	45-02231-0025
DP301-D+REF	45-02241-0025
DP301-I+REF	45-02251-0025
DP304-D+REF	45-02261-0025
DP304-I+REF	45-02271-0025
DP307-I+REF	45-02281-0025
DP310-I+REF	45-02291-0025
DPSRY+REF	45-02301-0025

Table 21 Ordering information for the allele-specific Mentype® DigitalQuant Assays, these assays are made for you upon request (on-demand ordering)

Assay	25 reactions
DP67-D+SRY	45-02311-0025
DP70-D+SRY	45-02321-0025
DP70-I+SRY	45-02331-0025
DP88-D+SRY	45-02341-0025
DP88-I+SRY	45-02351-0025
DP97-I+SRY	45-02361-0025
DP101-D+SRY	45-02371-0025
DP101-I+SRY	45-02381-0025
DP104-D+SRY	45-02391-0025
DP104-I+SRY	45-02401-0025
DP105-D+SRY	45-02411-0025
DP105-I+SRY	45-02421-0025
DP106-I+SRY	45-02431-0025
DP114-D+SRY	45-02441-0025
DP114-I+SRY	45-02451-0025
DP128-D+SRY	45-02461-0025
DP131-I+SRY	45-02471-0025
DP133-I+SRY	45-02481-0025
DP134-I+SRY	45-02491-0025
DP140-I+SRY	45-02501-0025
DP152-D+SRY	45-02511-0025
DP163-D+SRY	45-02521-0025
DP163-I+SRY	45-02531-0025
DP301-D+SRY	45-02541-0025
DP301-I+SRY	45-02551-0025
DP304-D+SRY	45-02561-0025
DP304-I+SRY	45-02571-0025
DP307-I+SRY	45-02581-0025
DP310-I+SRY	45-02591-0025

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Explanation of Symbols

	Manufacturer
RUO	For research use only. Not for use in diagnostic procedures.
	Batch code
	Contains sufficient reagents for <N> tests
	Consult electronic instructions for use (eIFU)
	Use-by date
	Temperature limit
	Catalogue number
	Keep away from sunlight
	Keep dry

Further marking used in this handbook:

	
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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