

Mentype[®] **DigitalQuant**

Sensitive and accurate absolute quantification

Instructions for Use

For research use only

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Biotype GmbH
Moritzburger Weg 67
D-01109 Dresden
Germany

*"xx" defines the specific DIP loci of the Mentype[®] **DigitalQuant** assays

Made in Germany

Biotype GmbH develops, produces, and distributes PCR-based applications for medical diagnostics. Our Mentype® test kits guarantee highest quality standards.

We are at your disposal for further information and suggestions.
Contact us or visit our website www.biotype.de.

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Mentype® DigitalQuant Amplification Kit

For research use only

Sensitive and accurate absolute DNA quantification

1. Product Description

1.1 Intended Use

The Mentype® **DigitalQuant** test kits are intended for the absolute quantification of DNA proportions from mixed samples.

The test kits are intended to be used for research purposes only and may not be used for diagnostic purposes.

The test kits should only be used by professional users trained in molecular biology techniques in general and in performing digital PCR in particular.

1.2 Overview and Explanations

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 β -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.

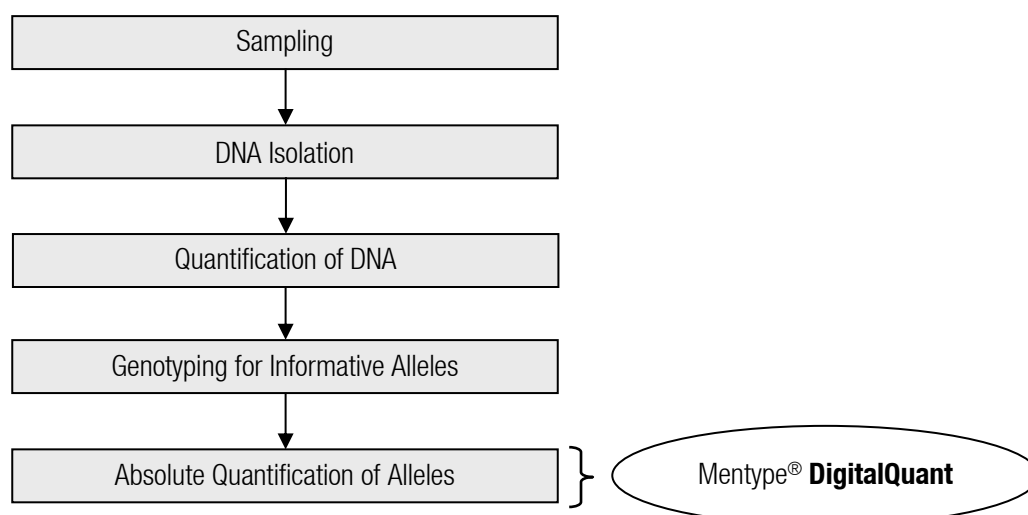


Figure 1 From sample to result using Mentype® **DigitalQuant**

Thereby, all specific DIP-Markers (see Table 16, page 22) are labeled with FAM. Accordingly, the active reference (REF) and the SRY Y-chromosome specific markers are detected in the HEX channel (for QuantStudio™ 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.

1.3 Platform and Software

The Mentype® **DigitalQuant** test kits have been validated with the Bio-Rad QX100™ and QX200™ Droplet Digital™ PCR System including the Quanta™Soft software (version 1.7.4) and the following thermal cycler:

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

The Mentype® **DigitalQuant** test kits have been verified for use on the Thermo Fisher Scientific Inc. QuantStudio™ 3D Digital PCR platform using the Thermo Fisher Scientific Inc. ProFlex™ 2x Flat PCR System Thermocycler. Verification was performed using the QuantStudio™ 3D AnalysisSuite™ Software (V3.1, Thermo Fisher Scientific Inc.) software. The application and performance of the Mentype® **DigitalQuant** kits on these devices must be independently validated by the user.

The use and performance of Mentype® **DigitalQuant** assays on instruments not mentioned above are the responsibility of the user and must be self-validated.

2. Delivered Materials

2.1 Kit Content

The following reagents for performing the Mentype® **DigitalQuant** assay are included in a kit:

Table 1 Content of a Mentype® **DigitalQuant** kit (25 reactions)

Component	Number of Vials	Vol./Vial [µL]	Cap Color	Storage
Nuclease-free Water	1	1,500	Blue	-25 °C to -15 °C
Primer Mix	1	63	Red	-25 °C to -15 °C
Mentype® DigitalQuant				

2.2 Ordering Information

Please direct your order to:

Email: sales@biotype.de

Fax: +49 (0) 351 8838 403

Table 2 Order information of Mentype® **DigitalQuant** assays

Product Name	Product Size	Order Number
Mentype® DigitalQuant	25 reactions	45-02xx1*-0025

*"xx" specifies the respective DIP of the Mentype® **DigitalQuant** assays.

Assay specific order numbers you can find in Table 18 and Table 19, page 24-25.

3. Warnings and Safety Instructions

3.1 Safety Instructions

Please observe the safety data sheet.

For the kit components, the safety data sheets are available on request. For safety data sheets of reagents that are not included in the test kit, please contact the respective manufacturer.

3.2 Quality Assurance

All kit components undergo an intensive quality assurance process at Biotype GmbH. The quality of the test kits is permanently monitored in order to ensure unrestricted usability. Please contact us if you have any questions regarding quality assurance (support@biotype.de).

3.3 Trademarks and Patents

Mentype® is a registered trademark of Biotype GmbH.

3.4 Storage and Handling of Reagents

Store the kits Mentype® **DigitalQuant** protected from light at -25 °C to -15 °C and avoid excessive freeze-thaw cycles (10 freeze-thaw cycles should not be exceeded).

The expiry date is indicated on the kit cover.

4. Implementation of Mentype® DigitalQuant – General Information

4.1 Sampling and Sample Handling

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

Sampling can take place using standard blood collection tubes. Samples should be stored refrigerated (4-8 °C) after collection and used for gDNA extraction as soon as possible.

For gDNA extraction, commercially available kits for the isolation of genomic DNA, e. g. by provider Qiagen or Machery-Nagel, may be used.

Concentrated gDNA can be adjusted to the required concentration by dilution with 1x TE buffer. gDNA should be stored at -20 °C ± 5 °C.

4.2 Optional: Determination of Allelic State

If the allele status of the informative loci is not known, it must be determined before calculating the gDNA 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 heterozygous in the marker.

If the ratio is **greater than 65 %**, it is a homozygous marker. The resulting calculation formulas can be found in the following Table 12 – Table 15.

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

4.3 Quantification Protocol

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.

5. Implementation using the QX100™ / QX200™ platform

5.1 Additional required reagents and consumables

Additional reagents are required to run the Mentype® **DigitalQuant** assays on the ddPCR platform QX100™/QX200™:

Table 3 Additional required reagents for Mentype® **DigitalQuant**

Reagent	Supplier
DIP Positive Control (DPC) (Order no.: 00-10030-0100)	Biotype GmbH
FastDigest <i>EcoRI</i> (e.g. Thermo Scientific™)	e. g. Fisher Scientific GmbH
ddPCR™ Supermix for Probes (No dUTP)	Bio-Rad Laboratories

Table 4 Standard reagents and devices of the QX100™/QX200™ system

Reagents	Supplier
QX100™/QX200™ Droplet Generator	Bio-Rad Laboratories
PX1 PCR Plate Sealer	Bio-Rad Laboratories
QX100™/QX200™ Droplet Digital™ PCR System	Bio-Rad Laboratories
Droplet Generation Oil for Probes	Bio-Rad Laboratories
ddPCR™ Droplet Reader Oil	Bio-Rad Laboratories
DG8™ Cartridge Holder	Bio-Rad Laboratories
DG8™ Cartridges for QX200™/QX100™ Droplet Generator	Bio-Rad Laboratories
DG8™ Gaskets for QX200™/QX100™ Droplet Generator	Bio-Rad Laboratories
PCR Plate Heat Seal, foil, pierceable	Bio-Rad Laboratories
ddPCR™ 96-Well Plates	Bio-Rad Laboratories
96-well PCR foil	Any

5.2 Mentype® DigitalQuant Master mix preparation

Table 5 below shows volumes of reagents for 5 µL of sample gDNA in a total reaction volume of 20 µL. The gDNA concentration should be 20 ng-60 ng in a maximum volume of 7.5 µL per assay.

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

Table 5 Master mix approach of one Mentype® **DigitalQuant** reaction using 5 µL gDNA

Components	Volume per reaction
Nuclease-free Water	2.5 µL
2 x ddPCR™ Supermix for Probes (No dUTP)	10.0 µL
Mentype® DigitalQuant Primermix	2.0 µL
FastDigest <i>EcoRI</i> Enzym	0.5 µL
Master mix volume	15.0 µL
gDNA (10 ng/µL)	5.0 µL

All components should be mixed (vortexed) and centrifuged for approximately 10 s before preparing the master mix.

When calculating the required mastermix volume, consider the number of positive and negative control reactions. Add one or two reactions to this number to compensate for pipetting errors.

5.2.1. Positive control (order separately)

Use 5 μL of DIP Positive Control (5 ng/ μL , Biotype GmbH) instead of template gDNA as a positive control for the duplex assay.

5.2.2. No template control

Nuclease-free water serves as no template control for the assay. Pipette respective volume instead of the gDNA template.

5.3 Restriction Digest

After transfer of PCR-Master mix containing sample gDNA and controls to the plate, close the plate with a PCR foil (not provided), mix thoroughly (vortex) and centrifuge briefly. Then incubate for **10 min at 37 °C** in a thermal cycler for the restriction digest.

Note: gDNA amounts of >100 ng of gDNA per ddPCR must be digested with EcoRI before the PCR reaction. gDNA amounts up to 100 ng of DNA per well can be digested directly in the PCR reaction setup.

5.4 Preparing the Droplet Digital PCR - Droplet Generation

Place the DG8 Cartridge in the Cartridge Holder. Pipette each sample (20 μL of digested PCR mix) up and down about 3 times before transferring the sample to the center row of the DG8 cartridge - observe Bio-Rad's general guidelines for droplet generation.

Start by pipetting the digested PCR mix. Transfer the samples in the DG8 cartridge from left to right. All 8 wells of the DG8 Cartridge must be filled with either sample or 1x ddPCR™ Buffer Control for Probes (not included in the kit).

After transferring all 8 samples, add 70 μL Droplet Generation (DG) Oil to the bottom row of the DG8 Cartridge. All 8 oil wells of the DG8 cartridge must contain DG oil.

Clamp the gasket over the DG8 cartridge by using the holes on both sides.

Note: When filling the DG8 Cartridge, always use the holder provided. The DG oil must first be distributed into the 8 wells of the DG8 cartridge when all 8 wells have been filled with sample.

Place the filled DG8 cartridge, still in the Cartridge Holder, into the QX100™/QX200™ Droplet Generator Instrument.

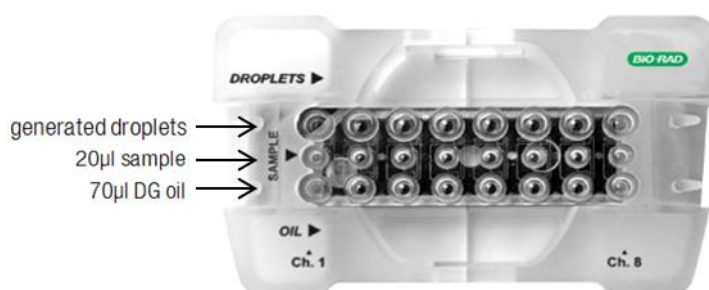


Figure 2 Cartridge holder with clamped cartridge showing the position of PCR sample (20 μL sample), Droplet Generation Oil (70 μL DG oil) and processed droplets

After droplet generation the top wells of the cartridge contain droplet-samples. Transfer 40 μL of the droplet-samples into a ddPCR™ 96-Well Plate.

Tip: Use an 8-channel pipette to save time.

Pipette the other samples into new cartridges.

Note: Handle samples carefully after droplet formation (do not vortex, do not centrifuge).

Seal the 96-well PCR plate with Pierceable Foil Heat Seal and place the plate into the PX1 PCR Plate Sealer. Also see the Bio-Rad instructions in the PX1 PCR Plate Sealer Instruction Manual (bulletin 10023997).

5.5 Digital PCR amplification

When heat sealing is completed, place the 96-well PCR plate into a thermal cycler and start the program according to Table 6.

Note: Do not vortex or spin down the plate.

Table 6 Digital PCR amplification parameters for Mentype® DigitalQuant

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

*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 **can not determine the block-material** use a ramp rate of **1 °C/s**.

5.6 Read droplets and analyze results

After the amplification is complete, place the PCR plate containing the sample droplets in the holder of the QX100™/QX200™ Droplet Digital™ PCR System (Droplet Reader).

Note: Do not vortex or spin down the plate!

5.6.1. Create the plate layout

Open the software Quanta™Soft from Bio-Rad. Create the plate layout for your experiment according to your sample scheme. 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.

To analyze the Mentype® DigitalQuant assay, use the settings listed in Table 7:

Table 7 Settings to be determined for analysis of the Mentype® **DigitalQuant** kit in the Quanta™Soft software

Sample and experiment types	Settings
Sample	
Name	give a name
Experiment	Absolute Quantification (ABS)
Supermix	ddPCR™ Supermix for Probes (no dUTP)
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 16) are labeled with FAM. The reference (REF) or SRY is marked accordingly with HEX.

After definition of the experiment click Run. The droplet reader counts fluorescence-positive and negative droplets for 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 are used for the concentration calculations

5.6.2. Analysis

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 3).

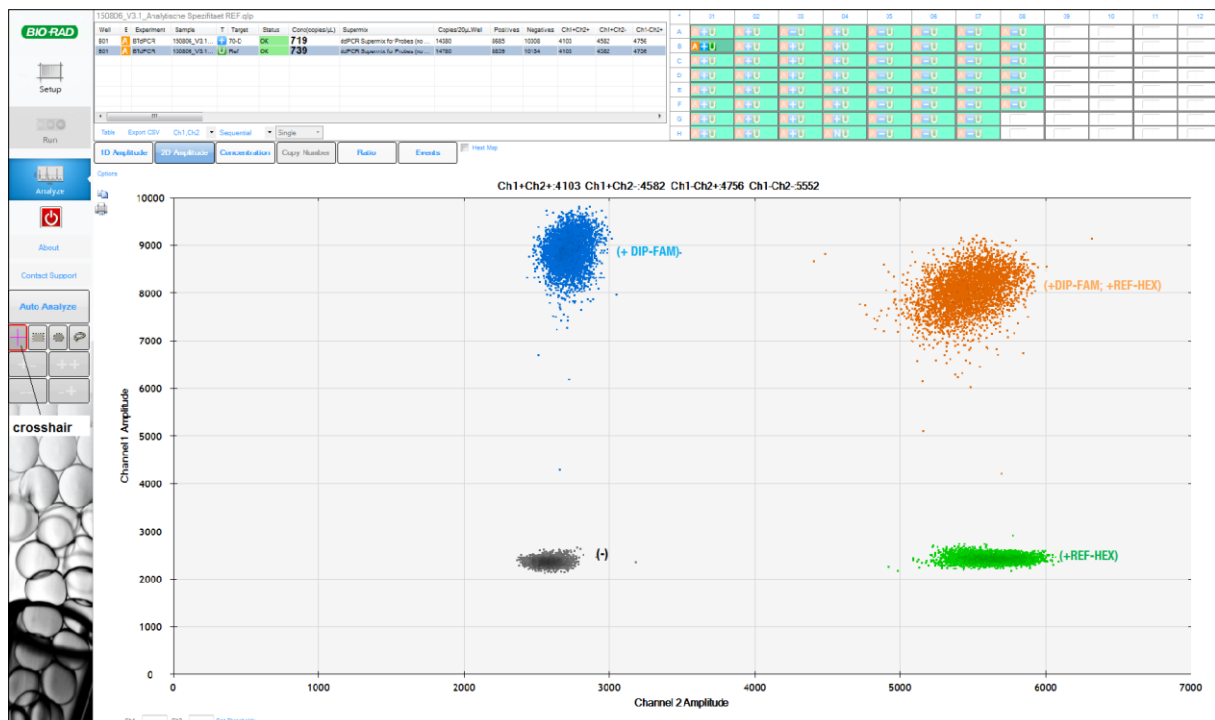


Figure 3 2D amplitude view (scatter plot) of the droplet fluorescence

All 4 cluster (grey, green, brown, blue) need to appear fully separated (see Figure 3 above). 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.

5.7 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

6. Implementation using the platform QuantStudio™ 3D

6.1 Additional reagents and equipment required

The following reagents are required to run the Mentype® **DigitalQuant** assays on the QuantStudio™ 3D platform:

Table 8 Additional required reagents for Mentype® **DigitalQuant**

Reagents	Supplier
QuantStudio™ 3D Digital PCR Mastermix v2	Thermo Fisher Scientific Inc.
DIP Positive Control (DPC) (Order no.: 00-10030-0100)	Biotype GmbH

Table 9 Standard Reagents and Devices of the QuantStudio™ 3D System

Reagents and devices	Supplier
QuantStudio™ 3D DigitalPCR Chip Kit v2	Thermo Fisher Scientific Inc.
QuantStudio™ 3D Digital PCR Instrument with Power Cord	Thermo Fisher Scientific Inc.
QuantStudio™ 3D Digital PCR Chip Loader	Thermo Fisher Scientific Inc.
QuantStudio™ 3D Digital PCR Chip Adapter Kit for Flat Block Thermal Cycler	Thermo Fisher Scientific Inc.
ProFlex™ 2x Flat PCR System	Thermo Fisher Scientific Inc.

6.2 Preparation of the Mentype® **DigitalQuant** master mix

Table 10 below shows the volumes of reagents needed for 5 µL of sample DNA in a total reaction volume of 14.5 µL. The gDNA concentration should be **50-60 ng** in a maximum volume of 8.7 µL per reaction.

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

Note: DNA amounts of >100 ng gDNA per dPCR assay should be digested with EcoRI before the PCR reaction.

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 10 Master mix approach for Mentype® **DigitalQuant** using 5 µL DNA

Component	Volume per reaction
Nuclease-free Water	3.7 µL
QuantStudio™ 3D MasterMix v2	4.3 µL
Mentype® DigitalQuant Primermix	1.5 µL
Master mix volume	9.5 µL
gDNA (10 ng/µL)	5.0 µL

All components should be mixed (vortexed) before preparing the master mix and centrifuged for about 10 s

Note: Prepare the reaction mixture completely for all samples. Addition of Mentype® **DigitalQuant** Primermix or gDNA during chip loading is not possible.

6.2.1. Positive control (not included in the kit)

Instead of DNA, use 5 µL of the DIP Positive Control (5 ng/µL, Biotype GmbH). It is recommended that at least one positive control be performed for each Mentype® **DigitalQuant** Assays used

6.2.2. No Template Control

Nuclease-free water serves as a negative control (no template control). Instead of DNA, pipette the appropriate volume of nuclease-free water to the reaction mixture. It is recommended that at least one negative control be performed for each Mentype® **DigitalQuant** assay used.

6.3 Loading the QuantStudio™ 3D Chips

For detailed information on loading the QuantStudio™ 3D Chips, refer to the manufacturer's instructions for use

- Turn on the QuantStudio™ 3D Digital PCR Chip Loader and wait for the instrument to be ready for use (LED solid green).
- Prepare the required QuantStudio™ 3D Chip Kit v2 consumables (chips, lids, blades, immersion oil sprays, tips included) and the required laboratory supplies.
- Remove the chip from the outer packaging, being careful not to touch the black loading area of the chip.
- Clamp the chip into the intended position of the QuantStudio™ 3D Digital PCR Chip Loader.
- Remove the chip lid (lid) of the outer packaging, being careful not to touch the glass surface of the lid.
- Remove the protective film from the surrounding adhesive surface and clamp the lid in the intended position of the QuantStudio™ 3D Digital PCR Chip Loader.
- Remove a loading blade from the outer packaging and also clamp it to the intended position of the QuantStudio™ 3D Digital PCR Chip Loader.
- Using a pipette, remove 14.5 µL of the prepared reaction mixture and pipette it into the sample loading port of the loading blade.
- Make sure that the reaction mixture is pipetted into the sample reservoir without bubbles and evenly distributed.
- Press the touchpad of the QuantStudio™ 3D Digital PCR Chip Loader to start loading the chip.
- While taking a syringe of immersion oil, open it and place the tip on the syringe.
- Discard the first drops of immersion oil.
- As soon as the loading of the chip is complete (the loading blade is no longer in contact with the chip), completely wet the black chip surface with immersion oil.
- Wrap the arm of the QuantStudio™ 3D Digital PCR Chip Loader to glue the chip to the lid. Press the arm on the chip for about 20-30 seconds.
- Remove the sealed chip from the QuantStudio™ 3D Digital PCR Chip Loader and with one hand hold open the not yet glued surface between the chip and the cover (lower left corner of the chip).
- Fill the chip completely with immersion oil.
- Note that the chip is completely filled, but the immersion oil does not overflow.
- Then remove the remaining protective tape from the lid and seal the chip manually.
- Store the chip horizontally in the dark while other chips are being loaded.

Start the PCR immediately, but no more than 2 hours after completing the loading of the chips.

6.4 Amplification parameters

After filling and sealing all QuantStudio™ 3D chips, PCR must be performed immediately. Insert the loaded chips into the PCR cycler. Make sure that the lettering of the chips is readable for you (QR code in the direction of the display, lettering in the direction of the lid of the device). Cover the chips with the QuantStudio™ Thermal Pads and close the cycler. The amplification parameters can be found in the following Table 11:

Table 11 Amplification parameters for performing Mentype® **DigitalQuant** assays

Temperature	Time	Number of cycles	Ramping rate
95 °C	10 min	1x	1 °C/s
94 °C	30 s	40x	
55 °C	60 s		
98 °C	10 min	1x	
10 °C	∞	1x	

Note that the lid is heated to 70 °C and the sample volume is adjusted to 1 nL.

6.5 Reading the QuantStudio™ 3D Chips

After the end of the PCR, the chips can be read out. In order to minimize possible condensation droplets on the chip, the chips should be stored dry for about 20-30 minutes at room temperature in the dark. Any condensation droplets that may still be present can be gently wiped in one direction with a lint-free cloth. The chips should be read within 24 hours after the end of the PCR.

Turn on the QuantStudio™ 3D device, open the drawer, and insert a chip into the slot provided. Close the drawer. The device processes and analyzes the chip. While analyzing a chip, the next chip can already be read.

After completion of the analysis, the chips can be stored at room temperature, lying flat, dry and dark. Repeated analysis of the chips is possible, but fluorescence loss during storage is possible.

Note: All specific DIP markers (see Table 16) are labeled with FAM; SRY is marked FAM in the SRY + REF combination. The REF marker is always shown in the VIC channel.

6.6 Analysis of raw data

The data collected will be analyzed using cloud software from Thermo Fisher Scientific Inc. The software analyzes the number of positive and negative wells for each fluorophore of the sample. The determination of the starting concentration of the target DNA is determined by means of the Poisson algorithm via the software (concentration of the copies/μL).

You will find the software QuantStudio™ 3D AnalysisSuite™ Software V3.1 in the access protected area of the Thermo Fisher homepage at <https://www.thermofisher.com/myssso/loginDisplay>. In the dashboard, select the AnalysisSuite dPCR app. In the opening view, all already created projects are listed. For a better overview, a project should be created for each experiment.

You will find the software QuantStudio™ 3D AnalysisSuite™ Software V3.1 in the access protected area of the Thermo Fisher homepage at <https://www.thermofisher.com/myssso/loginDisplay>. In the dashboard, select the **AnalysisSuite dPCR** app. In the opening view, all already created projects are listed. For a better overview, a project should be created for each experiment.

6.6.1. Software preparation

Create a new project

To create a new project, click Create Project in the lower-right corner of the window, then name the new project clearly.

Import data

In the newly created project, you are automatically directed to the **Import Data** tab. Click the **Import from local source** button if the calculator is not directly connected to the QuantaSoft™ 3D Digital PCR Instrument. If there is a connection between the computer and the QuantaSoft™ 3D Digital PCR Instrument, you can import the data via the **Import chip(s)** button. Select the location of the data and import it.

Define Chips

In the **Define Chips** tab, you can edit the raw data. It is possible to assign the sample name or a sample number in the column Sample. In addition, in the Task column a special property can be entered, e. g. No Template Control. Make sure the pre-sets in the Rare Dye (FAM), Target (VIC), Target (FAM) columns must comply with the specifications of the assay

6.6.2. View of the data

Go to the **Review Data** tab to view and, if necessary, adjust the visualization of the raw data.

First select the desired chip in the overview in the left area by clicking on it. Double-click on the corresponding chip to open the detail view of the chip (see Figure 4). This view contains the chip number, sample name, assay name, and task. In the detail view of the chip you can see the rating of each compartment. To do this, move the mouse pointer over the desired position in the chip view.

The detail view also contains the number of filled cavities (16,616 in Figure 4) and the number of wells evaluated (14,434 in Figure 4). The cavities are evaluated via an internal quality factor and used accordingly for evaluation. For more information please contact the support of Applied Biosystems.

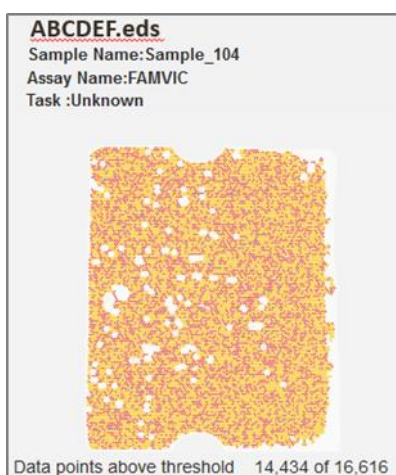


Figure 4 Detail view of a chip

In the right area of the window, the **2D view** of the chip opens (Figure 5), which can be opened at any time via the button. In this view, the results of the FAM channel are combined with those of the VIC channel. A point in this view corresponds to an evaluated compartment

- Yellow dots show no amplification of targets with FAM- and VIC-specific primers, the cavities are negative.
- Blue dots include amplification in the FAM channel but not in the VIC channel; here only the DIP loci used was amplified.
- Red dots contain amplification in the VIC channel but not in the FAM channel; only the REF or SRY loci were amplified here.
- Green dots indicate amplification of the FAM and VIC channels; Thus, both targets for the DIP loci and the REF or SRY loci were amplified.

In the upper area of the view, the absolute number of detected cavities per cluster is displayed.

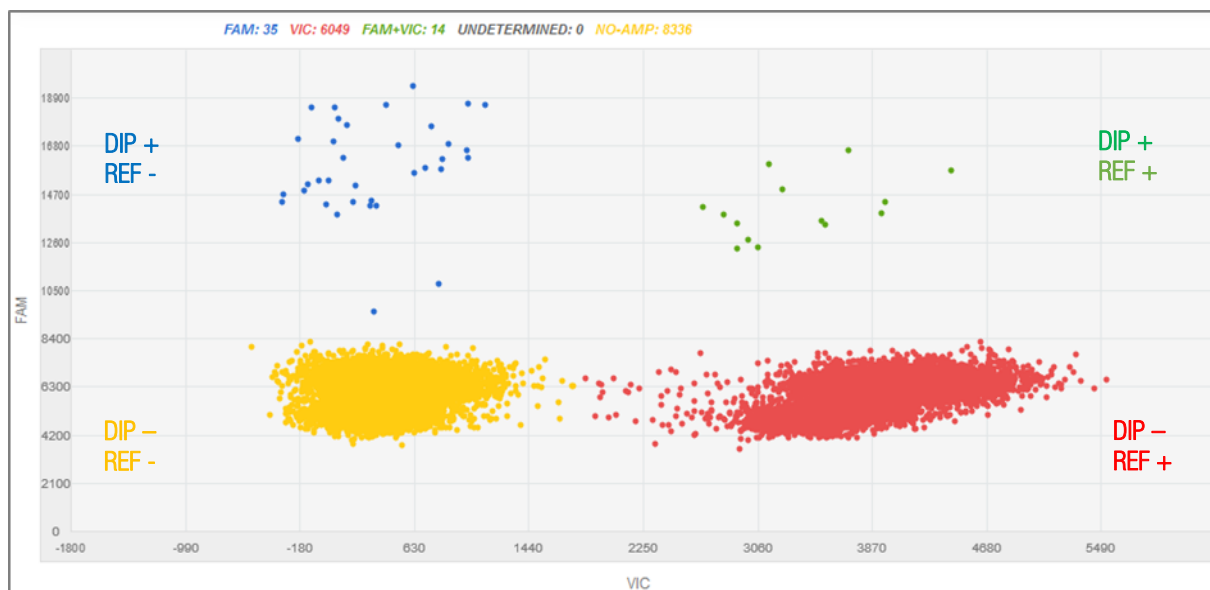


Figure 5 2D view of a chip with negative compartments (yellow), FAM-positive compartments (blue), VIC-positive compartments (red), and FAM and VIC-positive compartments (green)

Another helpful view is the **1D or Histogram view** of each color channel (Figure 6). Click on the button to switch to the histogram view. Open the histogram of the corresponding channel.

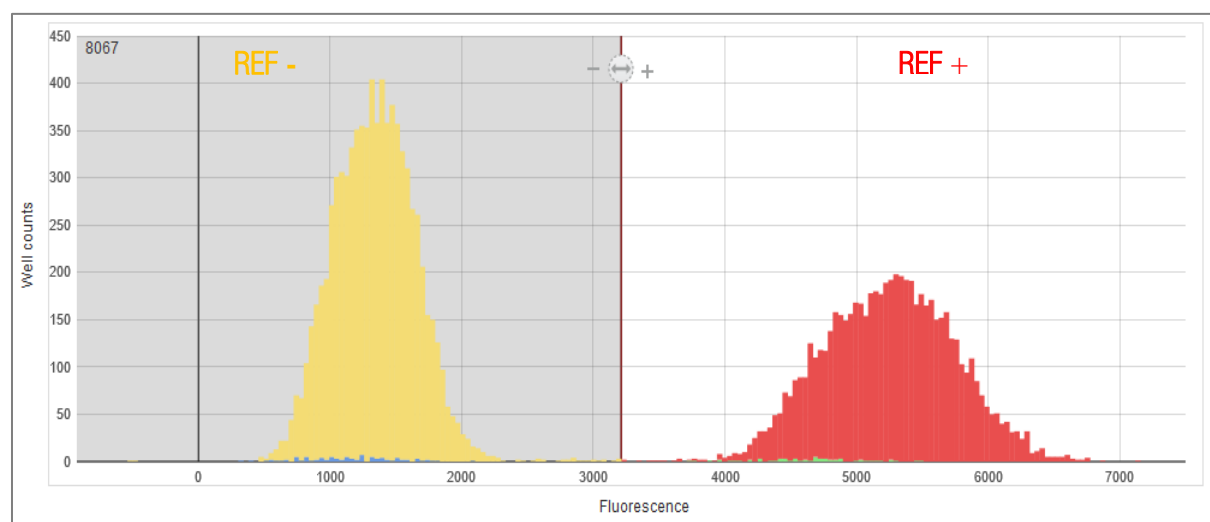


Figure 6 1D resp. histogram view of the chip's VIC channel, the bar between the maxima of the well counts can be moved to manually adjust the threshold

In both the 1D and the 2D view, the scales of the diagrams can be adjusted and set via the button

6.6.3. Adaptation of the data

The separation of the clusters may need to be adjusted. This can be done in the 1D or 2D view of the data. Each chip should be checked for a possibly necessary adjustment.

In the **1D view**, move the threshold bar (Figure 6) in the graph, or adjust the threshold in the appropriate window on the right.

In the **2D view**, use the tools on the right to assign data points to specific clusters.

6.6.4. Export of the data

After the raw data has been adjusted, switch to exporting the data as a .csv file to the **Export** tab. Export your **project data as CSV** and click Export. Save the file. Use the indicated concentrations Copies/ μ L (VIC) or Copies/ μ L (FAM) to calculate the chimerism value.

6.7 Minimum requirements for chip data before calculation

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

- At least 10,000 compartments should be included in the evaluation; the ratio of evaluated cavities to filled cavities should be over 70 %.
- There should be at least 5 positive compartments in the FAM channel to detect positive amplification. Here, the amplification in the FAM channel alone (blue cluster) and in the FAM and VIC-positive cluster (green cluster) are present. Samples with less than 5 positive wells in the FAM channel should be considered negative.
- The chips should be evenly loaded with sample and there should be no air bubbles on the chip (compare to the Troubleshooting section in the Thermo Fisher Scientific Inc. manual).

These values can be found in the exported data table in the **Replicates** section or in the 2D cluster (Figure 5) and the detail view of the chips (Figure 4).

Example

Using the sample from Figure 4 and Figure 5, one can assess the data quality as follows:

- 16,616 cavities were filled, 14,434 cavities were evaluated. The absolute number of evaluated cavities $14,434 > 10,000$, the loading of the chip is valid.
- The ratio of evaluated and filled cavities is $14,434 / 16,616 = 0.87 = 87 \%$, also this value is valid.
- A total of 35 positive cavities in the FAM channel and 14 positive cavities in the FAM + VIC channel were detected, so the values are above the limit of 5 positive amplifications.
- The chip has been loaded evenly; no anomalies are visible. The sample can therefore be calculated.

7. Calculation of absolute DNA ratios

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

Attention! 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** need be calculated as shown in Table 15.

Table 12 Calculation for DIP+Ref combination: homozygous AOI

Sample	AOI	SRY	Formula	Percent
	conc(copies)/ μL	conc(copies)/ μL		
Post-HSCT	338	644	$= (100 \cdot \text{AOI}) / (\text{REF})$ $= (100 \cdot 338) / 644$	52.5 %

Table 13 Calculation for DIP+Ref combination: heterozygous AOI

Sample	AOI	SRY	Formula	Percent
	conc(copies)/ μL	conc(copies)/ μL		
Post-HSCT	162	611	$= (100 \cdot 2 \cdot \text{AOI}) / (\text{REF})$ $= (100 \cdot 2 \cdot 162) / 611$	53.0 %

Table 14 Calculation for DIP+SRY combination: homozygous AOI

Sample	AOI	SRY	Formula	Percent
	conc(copies)/ μL	conc(copies)/ μL		
Post-HSCT	337	182	$= (100 \cdot \text{AOI}) / (\text{AOI} + 2 \cdot \text{SRY})$ $= (100 \cdot 337) / (337 + 2 \cdot 182)$	48.1 %

Table 15 Calculation for DIP+SRY combination: heterozygous AOI

Sample	AOI	SRY	Formula	Percent
	conc(copies)/ μL	conc(copies)/ μL		
Post-HSCT	553	506	$= (100 \cdot \text{AOI}) / (\text{AOI} + \text{SRY})$ $= (100 \cdot 553) / (553 + 506)$	52.2 %

8. Availability and characteristic of Mentype® DigitalQuant Assays

Table 16 Allele-specific Mentype® DigitalQuant Assays

Loci	Deletion (- Allele)	Insertion (+ Allele)	Allele-specific Duplex-Assay with Active 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	70-I	DP70-D+REF DP70-I+REF	DP70-D+SRY DP70-I+SRY
88	88-D	88-I	DP88-D+REF DP88-I+REF	DP88-D+SRY DP88-I+SRY
97		97-I	DP97-I+REF	DP97-I+SRY
101	101-D	101-I	DP101-D+REF DP101-I+REF	DP101-D+SRY DP101-I+SRY
104	104-D	104-I	DP104-D+REF DP104-I+REF	DP104-D+SRY DP104-I+SRY
105	105-D	105-I	DP105-D+REF DP105-I+REF	DP105-D+SRY DP105-I+SRY
106		106-I	DP106-I+REF	DP106-I+SRY
114	114-D	114-I	DP114-D+REF DP114-I+REF	DP114-D+SRY 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	163-I	DP163-D+REF DP163-I+REF	DP163-D+SRY DP163-I+SRY
301	301-D	301-I	DP301-D+REF DP301-I+REF	DP301-D+SRY DP301-I+SRY
304	304-D	304-I	DP304-D+REF DP304-I+REF	DP304-D+SRY 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	

Table 17 Locus-specific information and chromosomal localization

Locus	Chromosomal Localization	Locus	Chromosomal Localization
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		

9. References

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10. Technical Support

For specific product or technical support questions, please contact our Technical Support at:

Email: support@biotype.de

Phone: +49 (0) 351 8838 400

11. Order details of the Mentype® DigitalQuant Assays

Please direct your order to:

Email: sales@biotype.de

Fax: +49 (0) 351 8838 403

Table 18 Ordering information for the 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 19 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

Biotype GmbH
Moritzburger Weg 67
01109 Dresden
Tel. +49 351 8838 400
Fax +49 351 8838 403
info@biotype.de
www.biotype.de