



Digital PCR – QIAcuity One 5plex

General

QIAcuity systems are designed to determine absolute amounts of target DNA or RNA in a sample by using a digital PCR approach.

- absolute quantification
- mutation detection
- genome editing analysis
- copy number variation (CNV)
- gene expression analysis

- End-point PCR
- PCR reaction in many single partitions
- Amplicon sizes with an ideal range of 60bp-150bp
- Target molecule is detected by measuring the fluorescence
 - sequence specific DNA probes
 - or intercalating dyes
- Total number of filled partitions is identified by a passive fluorescent dye, present in the reaction mix
- Absolute quantification by dPCR eliminates the need of standard curves to determine amounts of target DNA in a given sample.
- Nanoplate-based system
- Workflow
 - Partitioning
 - Thermocycling
 - Imaging

Nanoplates



- Touch nanoplates only on the sides
- Place the nanoplate into the nanoplate tray to avoid damaging the optical surface and to keep it clean
- Do not centrifugate the nanoplate as this will lead to pre-priming and insufficient filling of the wells
- Do not vortex the nanoplate as this will lead to insufficient filling of the wells
- Transport the nanoplate horizontally
- You can reimage the plate e.g. if it was overexposed

Set up the QIAcuity Software

1. Login

For the QIAcuity PC

- user name -> QIAcuity PC
- password -> admin

For the QIAcuity software

- user name: admin
- password: admin

- to connect through the browser: <https://128.178.194.12:8687/Plates>

2. Create a new experiment

- Click new plate
- Enter plate name (please name it as such: PI_User_Date_XXX) and plate type
- Editing the plate barcode is optional
- **dPCR parameters:**
 - **Priming section:**
 - Select master mix
 - **Cycling section:**
 - Select cycling parameters (refer to master mix/probes user guide)
 - Enter each step and temperature
 - Steps can be grouped, edited or deleted
 - **Imaging section:**
 - unselect channels which are not required
 - Green channel: FAM, SYBR Green, EvaGreen
 - Yellow channel: VIC, HEX
 - Orange channel: TAMRA
 - Red channel: ROX
 - Crimson channel: Cy5
 - exposure time and gain settings are applied automatically
 - Depending on the assay different exposure time and gain settings might be required. Avoid oversaturation (warning appears during result analysis). *The plate can be imaged again after the run with different settings if needed.*
- **Reaction mixes**
 - Fill reaction mix name
 - Fill target name
 - Fill detection channel
 - Click create

- **Samples & controls**
 - Click new sample
 - Fill name and amount
 - Click create
 - For a bulk of samples set the amount to the sample amount needed: 3,4 etc
 - For controls and NT controls: enter name and add control
- **Plate layout**
 - Select reaction mix
 - Right click and select sample
 - Assign
 - Assign empty wells as blanc
 - Or for many samples import plate layout

Procedure

1. Template DNA fragmentation:

- Not required for DNA <20kb (e.g. cDNA, FFPE DNA or circulating DNA, cfDNA, or genomes < 20 kb)
- Restriction digestion is needed for DNA of average lengths of ≥ 20 kb (e.g., genomic DNA purified via spin columns with silica membrane)
- Selected restriction enzymes can be added into the reaction mix. EcoRI-HF, PvuII, XbaI (6-cutters), AluI, CviQI, HaeIII (4-cutters) digest template DNA in 10 minutes at room temperature in the QIAcuity PCR Master Mix
 - Ensure that the enzyme does not cut in the amplicon

2. Reaction set up using the QIAcuity EG PCR kit (Ref: 250111, 250112, 250113)

- The final volume depends on the nanoplate type used
- Never vortex or centrifuge the QIAcuity nanoplate

Component	Nanoplate 8.5 K (24-well, 96-well)	Nanoplate 26 K (24-well)	Final concentration
MasterMix	x μ l	x μ l	1x
10x primer mix or 10xprimer-probe mix	x μ l	x μ l	
<i>Restriction Enzyme (optional)</i>	<i>Up to 1 μl</i>	<i>Up to 1 μl</i>	<i>0.025-0.25 U/μl</i>
RNase-free water	variable	variable	
DNA (add in plate)	variable	variable	Must be in linear range of the plate (dilutions can be valuable).
Total reaction volume	12 μl	40 μl	

- a. Prepare the reaction mix corresponding to the MasterMix you are using (without the DNA template). *Restriction Enzyme is optional.*
- b. Vortex the mix
- c. Dispense appropriate volumes of reaction mix to tubes or the wells of a PCR plate

- d. Add DNA template
- e. Mix and vortex the tubes or PCR plate
- f. *Optional: incubate 10 minutes in case enzyme was added for template fragmentation*
- g. Handle the nanoplate carefully (never touch the bottom) and place it on a clean Nanoplate tray.
- h. Transfer the content of each well to the corresponding well of the nanoplate
- i. Make sure that no air bubbles are introduced into the wells of the dPCR plate during pipetting (bubble on top is less of a problem).
- j. Seal the nanoplate using the QIAcuity Nanoplate Seal
- k. Use the nanoplate roller 3-4 times vertically and horizontally
- l. Remove the transparent foil
- m. Use the nanoplate roller 3-4 times vertically and horizontally and then roll over the plate frame

3. Loading the plate into the QIAcuity instrument

- a. Transport of the Nanoplate horizontally and touch only the sides
It is recommended to cover the plate with the top seal within 30 minutes after pipetting to prevent subsequent filling issues. Run should be started directly (maximum 2 hours)
- b. Press the button on the instrument or the Eject Tray on the touchscreen.
- c. Place the plate in the tray
 - correct orientation: facing the barcode toward the instrument and the QIAGEN lettering toward you.
- d. The barcode of the loaded plate is scanned
 - Press the icon on the touch screen, and select an existing experiment defined in experiment
 - If the barcode does not match any plate in the software suite, use the control software to define the experiment. Enter Plate name etc.

Check quality of your images

- a. Click on the plate and select analysis
- b. If the image quality is good there are no warnings (proceed with result analysis)
- c. If exposure times or gains were too high, the image symbol will be highlighted in yellow. In this case you can reimage the plate with e.g. 30% lower exposure time:
 - i. Press the back button
 - ii. Select dPCR parameters
 - iii. Click on + and select + imaging
 - iv. Reduced exposure duration (eg. 700ms to 490ms)
- d. *Plate storage: Store plates at room temperature or in the fridge (without light). Plates can be reimaged still a few days later*

Results analysis

1. Select the selection you would like to use:

- Absolute Quantification
- Mutation detection
- Genome Editing
- Copy Number Variation
- Gene expression

2. e.g. for Absolute Quantification:

- Select the wells
- Select Target or detection channel
- Click show results
 - In list: the concentration, confidence interval, as well as positive and negative partitions are shown (Export as csv)
 - For an overview select heatmap: heatmap of the target channel will be shown
 - To change threshold values, select on heatmap, 1D scatterplot or 2D Scatterplot: Move the threshold or type in the value. Then click recalculate

3. How to handle errors

- Red color
- Click tools
- Select the red marked box
- Reboot instrument
- If a plate was aborted during a run the plate can be run again
- Save the plate and load it again in the instrument
- Edit and run to start

General notes

QIAcuity systems are intended to be used only in combination with QIAGEN kits indicated for use with the QIAcuity systems such as QIAcuity Nanoplates and QIAcuity PCR Reagents for the applications described in the kit handbooks.

If you would like to test other reagents with the QIAcuity, please contact GECF to discuss, but it is the user's responsibility to validate the performance.

Overview of the procedure

Troubleshooting

- Refer to the QIAcuity user manually, or contact us.

Changelog

- v1.01: initial release
- v1.02: many small changes
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