

Set-up, programming and analysis Mic qPCR Cyclers



General remark

Use **only assay templates programmed by Mikrogen** to perform *ampliCube* run analysis. Apart from the thermoprofile the templates contain assay-specific analysis parameters. Mic is considered as validated cyclor only when using the respective assay templates. Download at www.Mikrogen.de/downloads and type in “*ampliCube* Assay Templates for Mic”.

Additionally, assay templates for *alphaCube* are available at the link above, type in “*alphaCube* Assay Templates for Mic”. The use of assay templates for *alphaCube* is optional.



PCR reaction tubes for the Mic PCR cyclor must be **vortexed** for at least **10 sec at maximum speed** (recommended 3200 rpm).

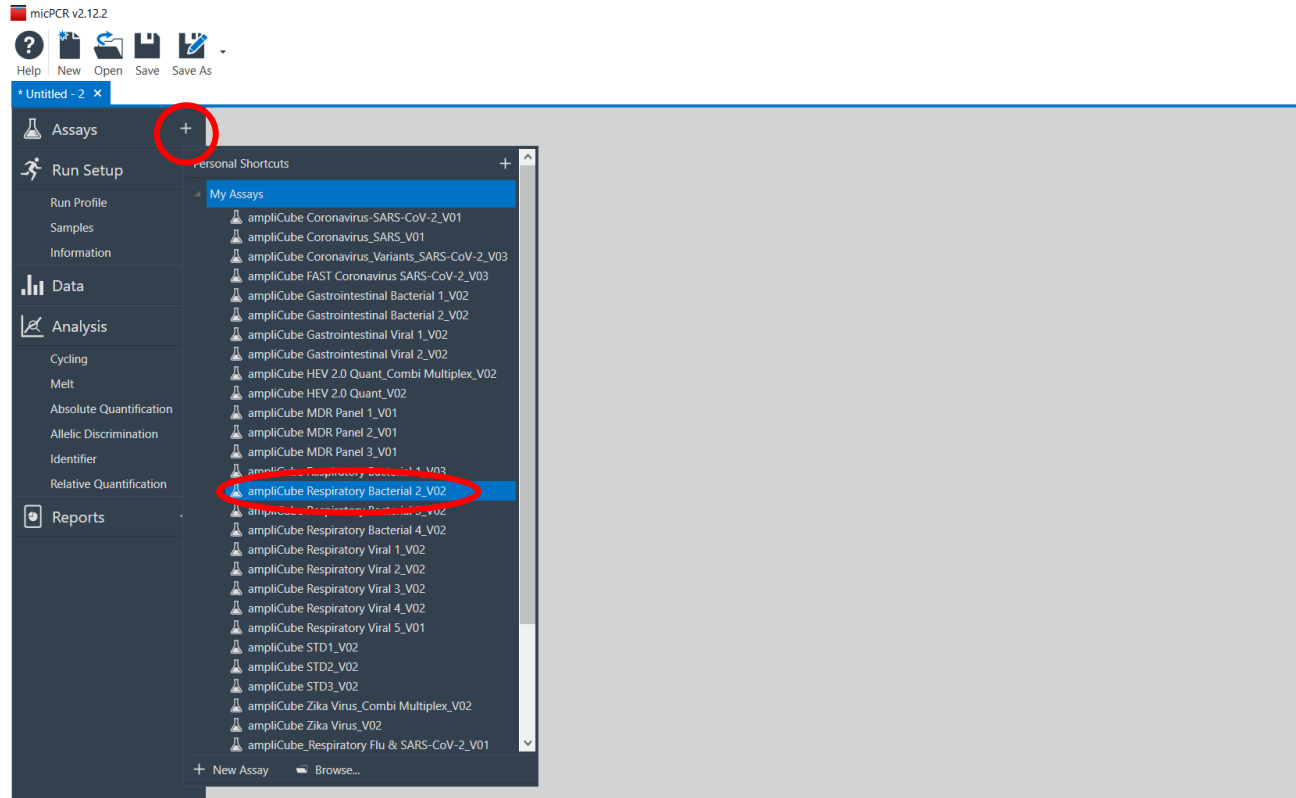
All descriptions in this setup manual are based on micPCR software version v.2.12.2.

How to start a run on Mic qPCR Cycler?



1. First start the Mic qPCR Cycler.
2. Then start the Mic software.
3. Choose „New“ and click on „Run“.

How to start a run on Mic qPCR Cycler?



1. Press „+“ next to Assay and select the assay you want to run.
2. If you intend to run several assays on the rotor, repeat step 1 and add the desired assays. If assay settings are not compatible because of different run profiles, a warning message will appear.

Instructions on how to import and save assays on the Mic is described in the Mikrogen manual „Import Assay Mic“.

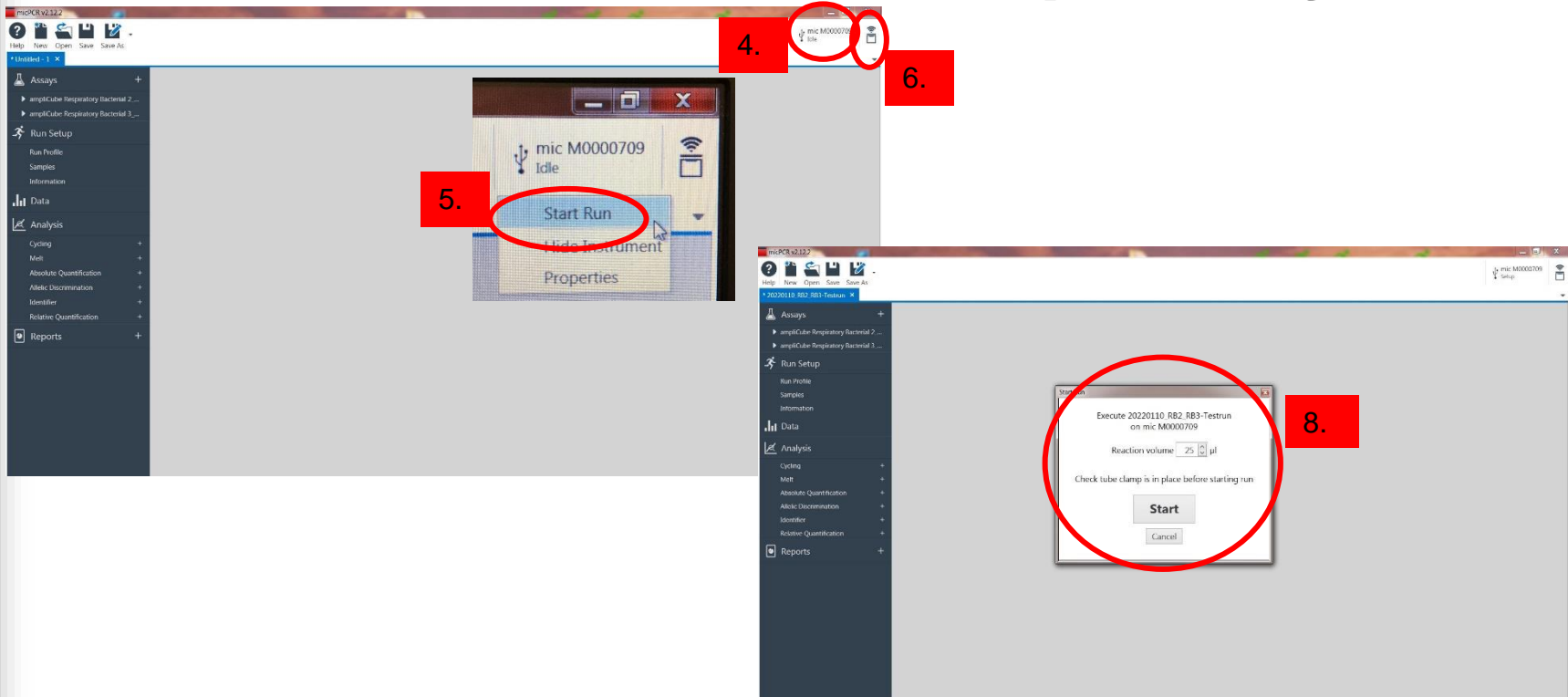
How to start a run on Mic qPCR Cycler?

The screenshot shows the micPCR v2.12.2 software interface. The sidebar on the left has 'Run Setup - Samples' selected, highlighted with a red box and the number '1.'. The main window displays a table of samples with columns: Colour, Name, Type, Assay, and Standards Concentration. The 'Name' column for the first sample is highlighted with a red box and the number '2.'. The 'Type' dropdown menu is open, showing options like 'Positive Control', 'Negative Control', 'Standard', and 'NTC', with a red box and the number '3.'. The 'Assay' column has a dropdown menu open, with a red box and the number '4.'. The 'Standards Concentration' field is highlighted with a red box and the number '5.'. A red circle highlights the 'auto fill' icon in the Assay column. The right-hand panel shows 'Available Assays' and 'Groups'. Labels '2b' and '4b' are placed above the Assay and Standards Concentration columns respectively.

Colour	Name	Type	Assay	Standards Concentration
1	Sample 1	Unknown	ampliCube Respiratory Bacterial 2_V02	
2	Sample 2	Unknown	ampliCube Respiratory Bacterial 2_V02	
3	Sample 3	Unknown	ampliCube Respiratory Bacterial 2_V02	
4	Sample 4	Unknown	ampliCube Respiratory Bacterial 2_V02	
5	Sample 5	Unknown	ampliCube Respiratory Bacterial 2_V02	
6	Sample 6	Unknown	ampliCube Respiratory Bacterial 2_V02	
7	Sample 7	Unknown	ampliCube Respiratory Bacterial 2_V02	
8	Sample 8	Unknown	ampliCube Respiratory Bacterial 2_V02	
9	Sample 9	Unknown	ampliCube Respiratory Bacterial 2_V02	
10	Sample 10	Unknown	ampliCube Respiratory Bacterial 2_V02	
11	Sample 11	Unknown	ampliCube Respiratory Bacterial 2_V02	
12	Sample 12	Unknown	ampliCube Respiratory Bacterial 2_V02	
13	Sample 13	Unknown	ampliCube Respiratory Bacterial 2_V02	
14	Sample 14	Unknown	ampliCube Respiratory Bacterial 2_V02	
15	PC Respi Bac 2	Positive Control	ampliCube Respiratory Bacterial 2_V02	
16	NC Respi Bac 2	Negative Control	ampliCube Respiratory Bacterial 2_V02	
17	Sample 15	Unknown	ampliCube Respiratory Bacterial 3_V02	
18	Sample 16	Unknown	ampliCube Respiratory Bacterial 3_V02	
19	Sample 17	Unknown	ampliCube Respiratory Bacterial 3_V02	
20	Sample 18	Unknown	ampliCube Respiratory Bacterial 3_V02	
21	Sample 19	Unknown	ampliCube Respiratory Bacterial 3_V02	
22	Sample 20	Unknown	ampliCube Respiratory Bacterial 3_V02	
23	Sample 21	Unknown	ampliCube Respiratory Bacterial 3_V02	
24	PC Respi Bac 3	Positive Control	ampliCube Respiratory Bacterial 3_V02	
25	NC Respi Bac 3	Negative Control	ampliCube Respiratory Bacterial 3_V02	
26		Standard		
27		Positive Control		
		Negative Control		
		NTC		

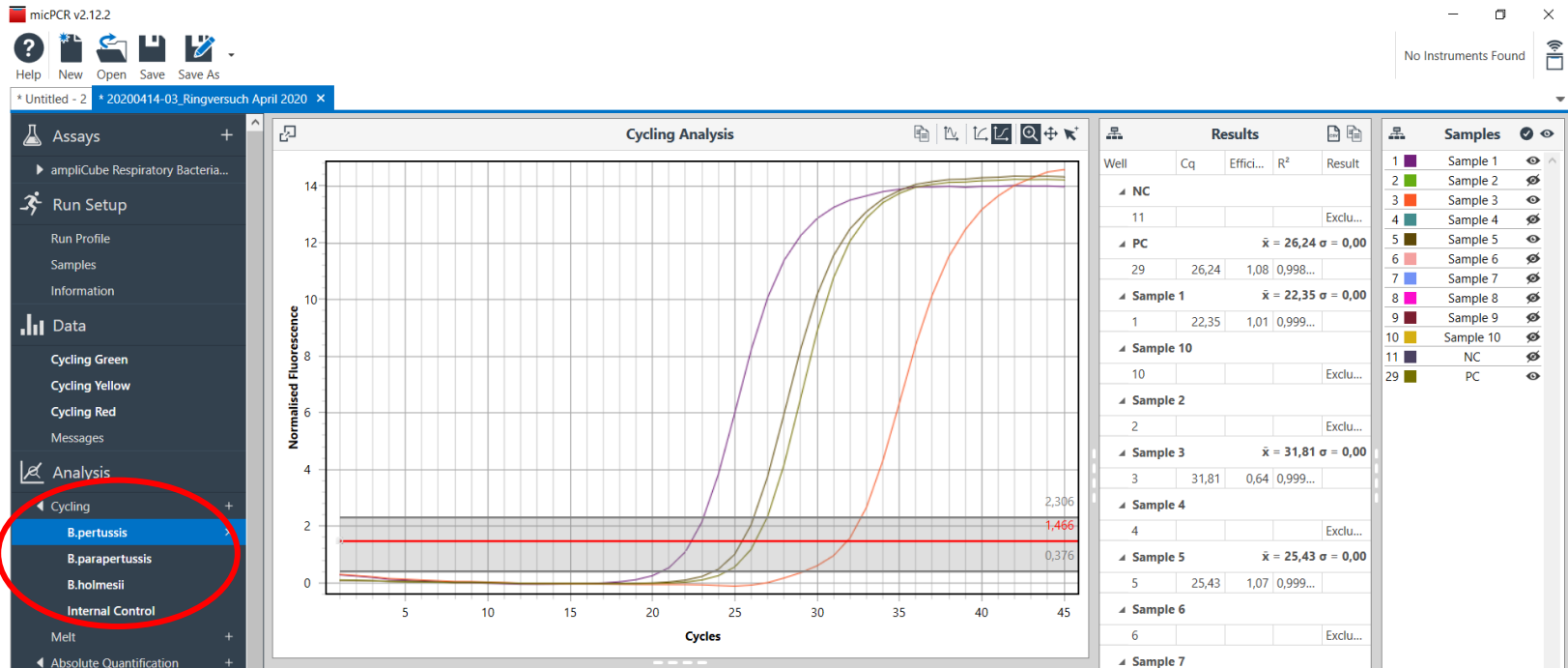
1. Select „Run Setup – Samples“ to open the sample editor.
2. Assign sample names manually or use a barcode reader. Use the „auto fill“ function, if convenient.
3. Define the „Type“ of sample (Positive Control, Negative Control, Standard if necessary).
4. In the field „Assay“ select the assay to be used from the drop down menu. One can use the „fill down“ function.
5. Add the concentration of standard in copies/μl, if you perform a quantitative analysis.

How to start a run on Mic qPCR Cycler?



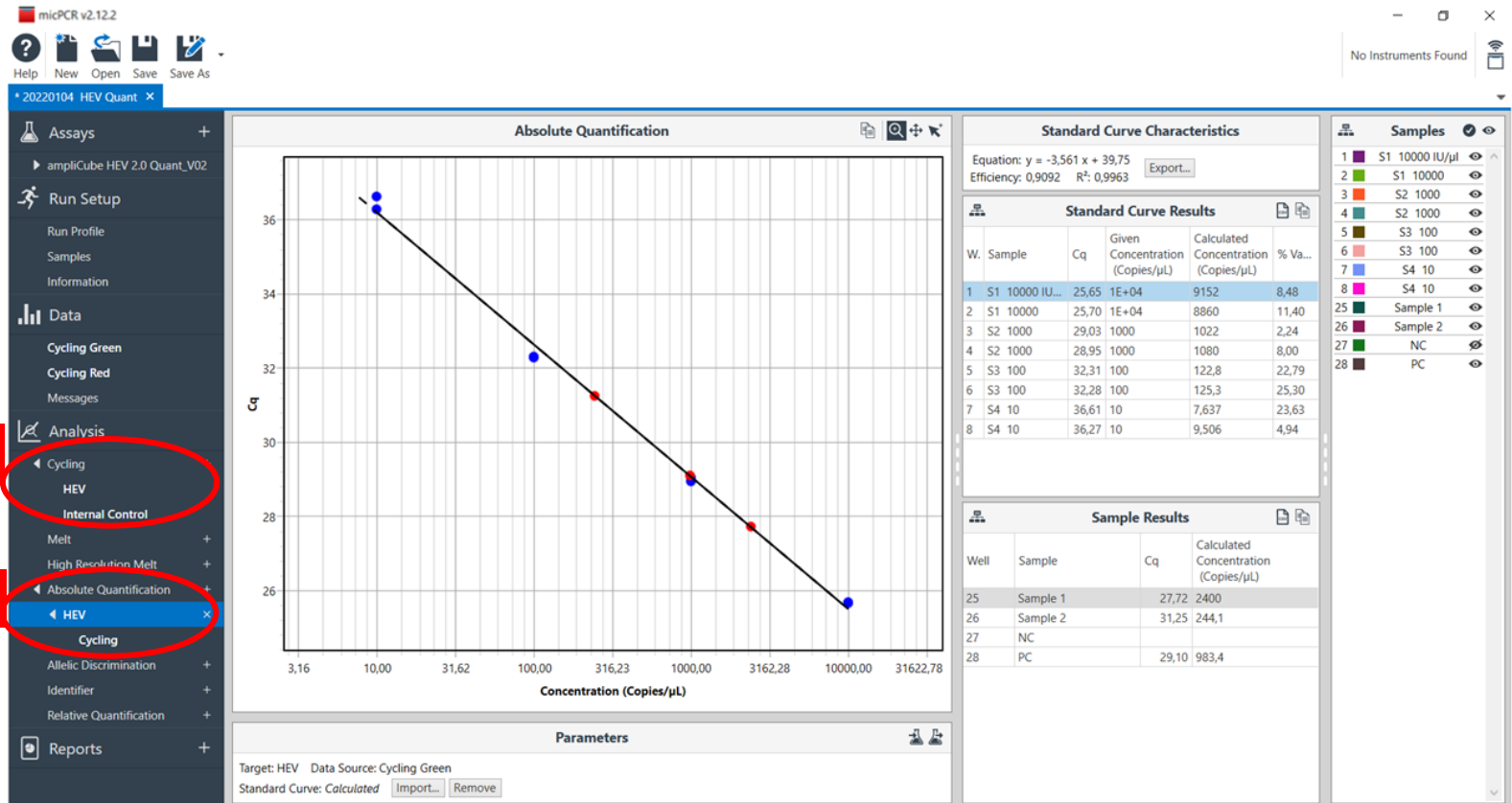
1. Place the **vortexed (10 sec at maximum vortex speed)** reaction tubes into the rotor keeping the tube tab in line with the marker located on the rotor label.
2. Load tubes filled with **water** into unused wells. Exchange these dummy tubes every 4-6 weeks. Never use old PCR reaction tubes as dummy tubes as they may influence future run analysis.
3. Add the tube clamp and close the lid.
4. Click on the instrument icon and choose „Start Run“ (5.). If instrument is not visible, click on the instrument icon (6.) and scan for instruments.
7. Save the run under your selected folder.
8. Confirm the reaction volume and start the run via the „Start“ button.

How to analyse a run on Mic qPCR Cycler?



1. In the analysis tab all targets are listed and an analysis per channel/assay is generated automatically after the run with the assay-specific parameters.
2. The graph and Ct-values will be shown. The software shows only the curves/Ct-values which are analysed positive by the software.
3. Go through all targets including the Internal Control and check the validity criteria of the respective IFU.

How to analyse a run on Mic qPCR Cycler? -quantitative-



1. In the analysis tab all targets are listed and an analysis per channel/assay is generated automatically after the run with the assay-specific parameters.
2. Results of quantification are shown in the tab „Absolute Quantification“.
3. The graph and Ct-values will be shown. Additionally, the results of the quantification standards are shown.
4. Check the validity criteria of the samples and standard curve as written in the respective IFU.

How to analyse a run on Mic qPCR Cycler?

The screenshot displays the micPCR v2.12.2 software interface. The left sidebar contains a 'Reports' icon (1). The top menu bar has a 'Save As' icon (2). The main window shows a 'Report' preview with 'Run Properties' and an 'Event Log'. The 'Results' table (3) is visible on the right, showing columns for Well, Cq, Efficiency, R², and Results. A 'Samples' list is also present on the far right.

Well	Cq	Effici...	R ²	Result
NC				
11				Exclu...
PC				
x̄ = 26,24 σ = 0,00				
29	26,24	1,08	0,998...	
Sample 1				
x̄ = 22,35 σ = 0,00				
1	22,35	1,01	0,999...	
Sample 10				
10				Exclu...
Sample 2				
2				Exclu...
Sample 3				
x̄ = 31,81 σ = 0,00				
3	31,81	0,64	0,999...	
Sample 4				
4				Exclu...
Sample 5				
x̄ = 25,43 σ = 0,00				
5	25,43	1,07	0,999...	
Sample 6				
6				Exclu...
Sample 7				

1. When all assays are analysed, export the results either by creating a pdf report (click on „+“ next to report in the left sidebar)
2. or export the data as Excel workbook (Save as - Excel workbook).
3. You can also copy your data table to the clipboard via the results tab or export it as a CSV file.



In case of questions, please contact
PCR.Support@mikrogen.de