qPCR Cycler Check™

Suitable for common real-time PCR cyclers

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

False negative PCR results or unspecific amplifications might be caused by a defect PCR cycler. Both events are highly critical for Good Laboratory Practise (GLP). Verification of the correct temperature control of the equipment in-use is usually a strenuous task, and the compliance with quality management systems is not easy to fulfil for PCR cyclers. Although electronic temperature sensors offered for purchase or as service usually measure the temperature homogeneity in a cycler block, this measurement does not necessarily reflect all parameters which need to be controlled by the cycler, and can therefore fail to provide reliable PCR results. Only a reference setup can investigate all relevant parameters of the process reliably.

The qPCR Cycler Check™ kit is designed for the verification of block or air-heated qPCR cyclers as part of the installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ), as required in various international norms, such as EN ISO 17025, EN 45001, EN ISO 13485, ISO/TS 20836:2007, GLP, GMP, and others.

TEST PRINCIPLE

qPCR Cycler Check™ is a thermal PCR cycler validation kit, which provides temperature sensitive PCR reactions to verify the temperature sensor and cycler processor in a realistic run. The primer sequences in combination with a regular PCR protocol were designed to react extremely sensitive to incorrect temperature control, temperature homogeneity, precision and timing. Amplification will be altered and indicated by different C_t values and morphology of the amplification curve at temperature differences of more than 2 °C. The cycler performance is tested at typically used PCR settings to reflect most user applications. In addition, the pre-adjusted target concentrations are only amplified at high PCR efficiencies as an additional indicator for accurate temperature control of the thermal cycler.

REAGENTS

The expiry date of the unopened package is marked on the package label. The kit components are stored at +2 to +8 °C until use.

Kit Component	Quantity	Cap color
Validation Mix	4 vials, 25 reactions each, freeze-dried	Red
Rehydration Buffer	2 vials, 1.9 ml	Blue
Control	1 vial, freeze-dried	Green

The lot specific Certificate of Analysis can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The qPCR Cycler Check™ kit contains all reagents required for the validation of thermal PCR cyclers. General industrial supplies and reagents, usually available in PCR laboratories, are required but not included:

- qPCR device with filter sets for the detection of the fluorescence dyes FAM and ROX
- PCR reaction tubes for the specific qPCR device
- 1.5 ml reaction tubes, DNA- and RNA-free
- Microcentrifuge for 1.5 ml PCR reaction tubes
- Pipettes with corresponding filter tips to prepare and dispense the reaction mix (10, 100 und 1000 μl)

PRECAUTIONS

qPCR Cycler Check™ is intended for use in research and quality control. It should be used by trained laboratory staff only. This kit does not contain hazardous substances and may be disposed of according to local regulations.

ADDITIONAL NOTES

- These instructions must be understood to successfully use the qPCR Cycler Check™ kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents must not be used beyond their shelf life.
- Follow the exact protocol. Any deviations may affect the test method and results.
- Additional control samples are not required. This kit already contains all controls necessary for the test.

TEST PROCEDURE

The validation reaction should be performed precisely as described. All reagents and samples must be equilibrated to +2 to +8 °C prior use.

1. Rehydration of reagents

1.	Spin down freeze-dried components, Validation Mix (red cap) and Control (green cap), for 5 sec at maximum speed.
2.	Add 700 µl Rehydration Buffer (blue cap) to each vial of the Validation Mix (red cap), and 100 µl to the Control vial (green cap).
3.	Close the vials and incubate for 5 min at room temperature.
4.	Vortex briefly and spin down for 5 sec.
5.	After reconstitution, reagents should be stored at ≤ -18 °C.

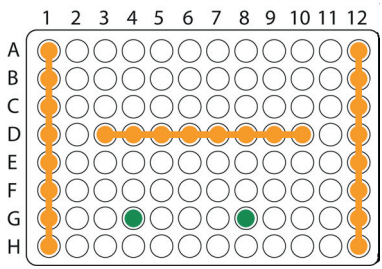
2. Preparation of the Reaction Tubes

1.	Validation reactions: Add 25 µl of Validation Mix (red cap) to each PCR tube (e.g. as indicated by the orange-colored positions in the diagram below).
2.	Control reactions: Prepare at least 2 vials per run, e.g. as indicated by the green-colored positions in the diagram below, by adding 23 µl of Validation Mix to the PCR tube and 2 µl of the Control (green cap).
3.	Close tightly and spin down all PCR tubes briefly.

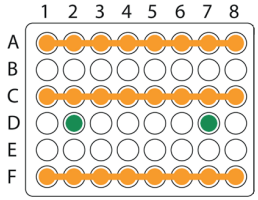
3. Loading the Tubes

The loading scheme depends on the block format of the PCR cycler to be tested. The following schemes are suggestions for regular testing. If particular peltier elements, segments of the block, or cavities are already subject of investigation, the strips or even individual tubes can be placed variably within the block.

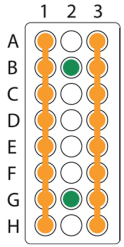
96 well block:



48 well block:



24 well block:



Legend	
Yellow positions	Validation reactions
Green positions	Control reactions

4. Starting the reaction

1.	Load the cycler, check each PCR tube and the cycler lid for tight fit.
2.	Program the qPCR cycler or check stored temperature profiles. See Appendix I for temperature profiles of selected qPCR cyclers. Programs for several additional cyclers are available upon request.
3.	Start the program and data reading.

5. Result reading

1.	Save the data at the end of the run.
2.	Read the channels for the wavelengths 520 nm and 610 nm and show the 2nd deviation of the data.
3.	Read the calculation of the C_t values for all reactions.
4.	Evaluate in accordance with the laboratory and instrument-specific reference ranges.

INTERPRETATION OF RESULTS

The cycler passes the test and the results of the check are valid if signals are visible in the ROX channel and comparable in C_t value and signal strength to the control reaction. The cycler does not comply with the expected specifications by either showing signals in both channels or no signal at all:

Channel FAM		Channel ROX		Result	Consequence for your standard application
Validation reactions	Control reactions	Validation reactions	Control reactions		
yes ($C_t < 35$)	yes ($C_t < 35$)	yes	yes	temperature too low	PCR conditions not stringent enough; risk of false positive results
no ($C_t > 35$)	yes ($C_t < 35$)	yes	yes	cycler ok	good results
no	no ($C_t > 35$)	yes	yes	temperature too high	PCR conditions too stringent; risk of low PCR efficiency / sensitivity
no	no	no	no	fatal error	no results

Please note, that all PCR reactions should show a uniform result. If not, most likely one or even more of the peltier elements show a malfunction. In this case, the experiment should be repeated with an adopted loading scheme. For amplification curves see page 11.

APPENDIX I

Programming and data recording devices of different qPCR

Rotor-Gene® 6000 (5-plex)

Please check the correct settings for the filter combination:

green filter (470-510): probe 1
orange filter (585-610): probe 2

Target	probe 1	probe 2
Channel	green	orange

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	40
Denaturation	95 °C for 15 sec
Annealing/Elongation	58 °C for 30 sec ⟶ acquiring to Cycling A (green and orange)
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and orange) according to the following table and start data analysis by double click.
- The following windows will appear:
  Quantitation Analysis - Cycling A (green or orange)
  Quant. Results - Cycling A (green or orange)
  Standard Curve - Cycling A (green or orange)
- In window *Quantitation Analysis*, select first *linear scale* and then *slope correct*
  - Threshold setup
  - In window *CT Calculation* set the threshold value to 0-1
  - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The C_t-values can be taken from the window *Quant. Results.*
- Samples showing no C_t-value can be considered as negative.

ABI Prism® 7500

Detector Settings:

Probe 1	Reporter - FAM / Quencher - none
Probe 2:	Reporter - ROX / Quencher - none

The ROX Reference needs to be disabled. Activate both detectors for each well. Measurement of fluorescence during extension.

Program Step 1: Pre-incubation

Setting	Hold
Temperature	95 °C
Incubation time	3:00 min

Program Step 2: Amplification

Cycles	40
Setting	Cycle
Denaturing	95 °C for 15 sec
Annealing/Extension	60 °C for 30 sec

Result Reading:

- Enter the following basic setting at the right task bar:
  Data: Delta RN vs. Cycle
  Detector: FAM and ROX
  Line Color: Well Color

	probe 1	probe 2
channel	FAM	ROX

- Open a new window with for the Graph settings by clicking the right mouse botton
  Select the following setting and confirm with ok:
  Real Time Settings: Linear
  Y-Axis Post Run Settings: Linear and Auto Scale
  X-Axis Post Run Settings: Auto Scale
  Display Options: 2
- Initiate the calculation of the C_t-values and the graph generation by clicking on Analyze within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no C_t-value can be considered as negative.

Mx3005P®

- Go to the setup menu, click on „Plate Setup“, check all positions which apply
- Click on „Collect Fluorescence Data“ and check FAM and ROX
- Corresponding to the basic settings the „Reference Dye“ function should be deactivated
- Specify the type of sample (negative or positive control, sample, standard) at „well type“
- Edit the temperature profile at „Thermal Profile Design“:
  Segment 1: 3 min, 95 °C
  Segment 2:
  Denaturing 95 °C for 15 sec
  Annealing/Extension 60 °C for 30 sec, data collection end
  40 cycles
- at menu „Run Status“ select „Run“ and start the cycler by pushing „Start“

Analysis of raw data:

- In the window „Analysis“ tab on „Analysis Selection / Setup“ to analyse the marked positions
- Ensure that in window „algorithm enhancement“ all options are activated:
  Amplification-based threshold
  Adaptive baseline
  Moving average
- Click on „Results“ and „Amplification Plots“ for an automatic threshold
- Read the C_t values at „Text Report“

CFX96 Touch™

Run Setup - Protocol Tab

- Click **Create New** to open the Protocol Editor to create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click the temperature or incubation time to directly edit the value.

PCR Program

	Step 1	Step 2	Step 3	Step 4
Temperature	95.0 °C	95.0 °C	60 °C	40 cycles
Incubation time	03:00 min	00:15 sec	00:30 sec	

Run Setup - Plate Tab

- Click **Create New** to open the Plate Editor to create a new plate.
- Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run. Important!!! Select the **All Channels** mode.
- Click the **Select Fluorophores** button to indicate the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

Run Setup – Start Run Tab

- View the selected Protocol file, Plate file, and data acquisition Scan Mode setting in the **Run Information** pane.
- Select one or more blocks and edit run parameters if necessary in Start Run on Selected Block(s) pane.
- Click the Start Run button to begin the run.

Data Analysis

Quantification Tab

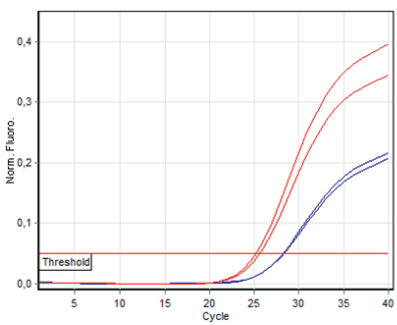
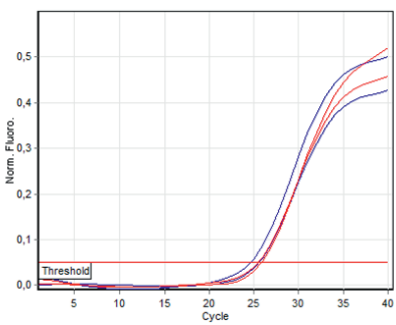
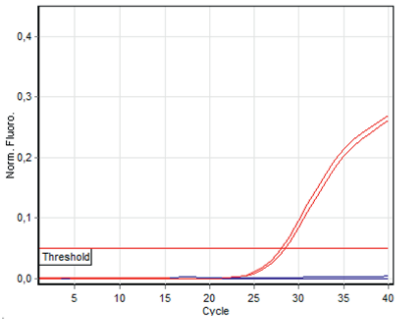
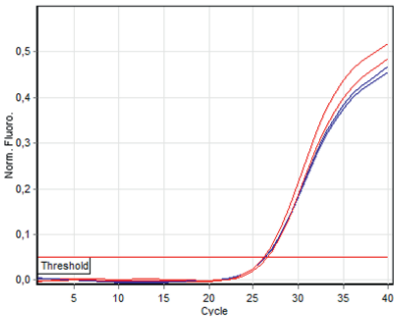
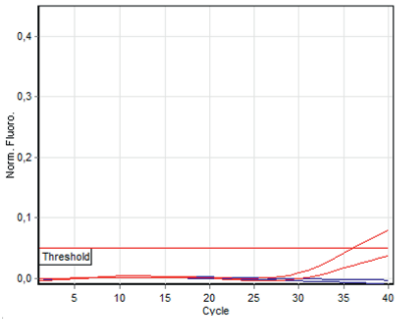
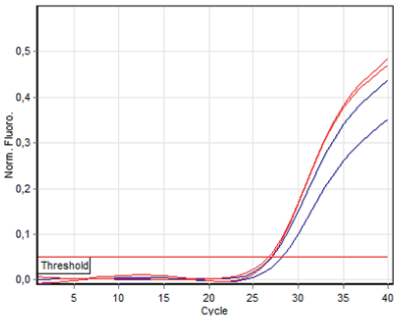
The amplification chart data in this tab display the relative fluorescence (RFU) collected from each well at every cycle of the run.

- Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart.

Data Analysis Settings

- The Software uses two modes for quantification cycle determination. Select **Settings** from the menu bar and select **Baseline Subtracted Curve Fit** as baseline setting and **Single Threshold Mode** as Cq Determination Mode.
- In the **Single Threshold Mode**, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

Results produced with a Rotor-Gene® 6000 qPCR device:

FAM Channel	ROX Channel	
		**temperature too low**
		**temperature o.k.**
		**temperature too high**

Legend	
Blue curves	Validation reactions
Red curves	Control reactions

APPENDIX II

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

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Related Products

Conventional PCR Cycler Validation kits

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions

ConviFlex™ DNAmp Mix

191-025/100/250	PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions

SwabUp™ Lab Monitoring Kits

181-0010/0050	Sample collection and DNA extraction	10/50 samples
182-0010/0050	Sample collection, DNA extraction and PCR system	10/50 samples

Food and Water Assays

11-02-XX-025	Food Control™ qPCR	25 reactions
12-01-005/-020/-040	Meat ID™ Screen	5/20/40 tests
12-02-025/-100	Meat ID™ Halal	25/100 reactions
12-05-025/-100	Vegan Control™	25/100 reactions
34-2025/-2100/-2250	AquaScreen® qPCR	25/100/250 reactions

Contamination Control Kits for conventional PCR

11-1025/1050/1100/1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/7048/7096/7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/8050/8100/8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/1050/1100/1250	Onar® Bacteria Detection Kit	25/50/100/250 tests

Contamination Control Kits for qPCR

11-9025/9100/9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 tests

Nucleic Acid Extraction

601-1010/-1050	ExtractNow™ DNA Mini Kit	10/50 extractions
602-1010/-1050	ExtractNow™ Blood DNA Mini Kit	10/50 extractions
603-1010/-1050	ExtractNow™ RNA Mini Kit	10/50 extractions
604-1010/-1050	ExtractNow™ CleanUp Kit	10/50 extractions
605-1010/-1050	ExtractNow™ Plasmid Mini Kit	10/50 extractions
606-1010/-1050	ExtractNow™ Virus DNA/RNA Kit	10/50 extractions

MB Taq DNA Polymerase

53-0050/0100/0200/0250	MB Taq DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/1100/1200/1250	MB Taq DNA Polymerase (1 U/µl)	50/100/200/250 units

PCR Clean™

15-2025	DNA Decontamination Reagent, spray bottle	250 ml
15-2200	DNA Decontamination Reagent, refill bottles	4 x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 Sachets

Lab Clean™

15-4100	Molecular microbiology lab cleaner, bottled	1 Liter

WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 x 5 ml/3 x 50 ml/500 ml

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