qPCR Cycler Check™

Suitable for common real-time PCR cyclers

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

False negative PCR results or unspecific amplifications might be caused by a defective PCR cycler. Both events are highly critical for Good Laboratory Practice (GLP). Verification of the correct temperature control of the equipment in-use is generally a strenuous task. Furthermore, compliance of PCR cyclers with quality management systems is not easy to achieve. Although commercially available temperature sensors or verification/calibration services can usually measure temperature uniformity in a cycler block, this measurement does not necessarily reflect all critical parameters for the accurate functioning of the cycler. Only a reference setup can reliably investigate all relevant parameters of the process.

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 by 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 be extremely sensitive to fluctuations in temperature and homogeneity, precision of the temperature control and timing.

Amplification will be altered when temperature deviates of more than 2 °C from the set value resulting in unexpected Ct values and morphology of the amplification curves. Cycler performance is tested with typical PCR settings to reflect most common applications. As an additional indicator of the accurate temperature control of the cycler, the included pre-adjusted target concentrations are only amplified by highly efficient PCRs.

CONTENT

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. Do not freeze or store the Validation Mix after reconstitution.

Component	Quantity	Cap color
Validation Mix	4 vials, 26 reactions each, lyophilized	Red
Rehydration Buffer	2 vials, 1.9 ml each	Blue
Positive Control	1 vial, lyophilized	Green

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

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 (10, 100 und 1000 µl)

PRECAUTIONS

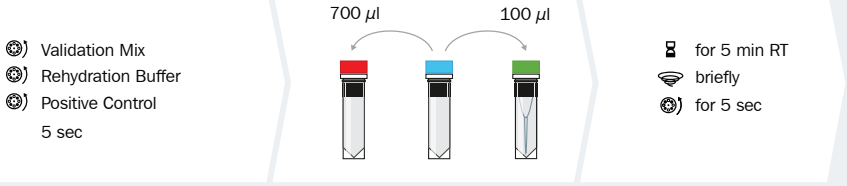
qPCR Cycler Check™ is intended for use in research and quality control. It should be used by trained laboratory staff only. This kit does not contain hazardous substances and may be disposed of according to local regulations.

ADDITIONAL NOTES

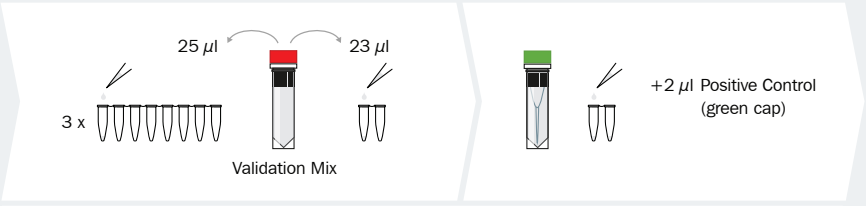
- These instructions must be understood to successfully use the qPCR Cycler Check™ kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents must not be used beyond their shelf life.
- Follow the exact protocol. Any deviations may affect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

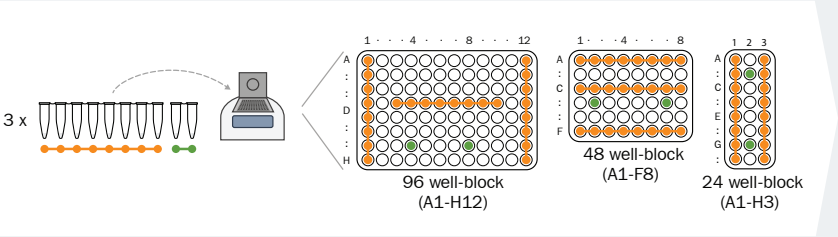
PROCEDURE – OVERVIEW

1. Reagents preparation

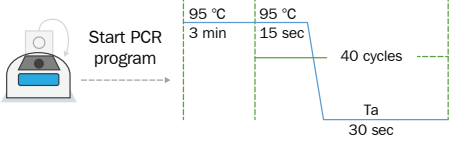


2. Preparation of the reaction tubes





3. qPCR amplification



4. Analysis

FAM™		ROX™		
●	●	●	●	**Results**
✓	✓	✓	✓	Temperature too low
✗	✓	✓	✓	Cycler ok
✗	✗	✓	✓	Temperature too high
✗	✗	✗	✗	Fatal device error

Validation Mix
Rehydration Buffer
Positive Control

Incubate
Vortex
Centrifuge
Ta Annealing Temperature

This procedure overview is not a substitute for the detailed manual.

MB_SI_qPCR_Cycler_Check_01_EN

PROCEDURE - STEP BY STEP

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

1. Rehydration of reagents

1.	Spin down lyophilized components, Validation Mix (red cap) and Positive 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 Positive Control vial (green cap). Please note: Do not store or freeze the rehydrated Validation Mix. Reconstitute only the vial(s) of Validation Mix necessary to carry out the selected number of reactions (e.g. 1 vial per 26 reactions, corresponding to 1 cycler validation).
3.	Close the vials and incubate for 5 min at room temperature.
4.	Vortex briefly and spin down for 5 sec.

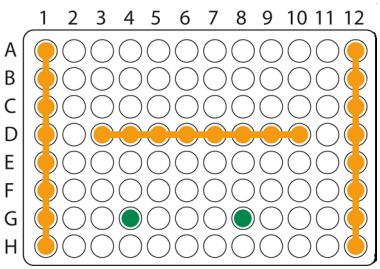
2. Preparation of the reaction tubes

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 PCR tubes per run, e.g. as indicated by the green-colored positions in the diagram below, by adding 23 µl of Validation Mix to each PCR tube and 2 µl of the Positive Control (green cap).
3.	Close tightly and spin down all PCR tubes briefly.

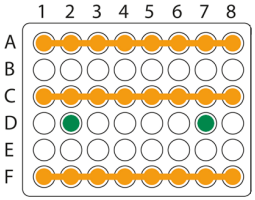
3. Loading the tubes in the cycler

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.

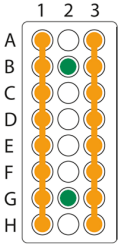
96 well block:



48 well block:



24 well block:



Legend	
Yellow positions	Validation reactions
Green positions	Control reactions

4. Starting the qPCR reaction

1.	Load the cycler, check each PCR tube and the cycler lid for tight fit.
2.	Program the qPCR cycler as follows or check stored temperature profiles. 1 cycle 95 °C for 3 min 40 cycles 95 °C for 15 sec T_a for 30 sec (Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA)) 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. Analysis

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.

DATA INTERPRETATION

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 (C_t > 35)	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 the test should show consistent results in all PCR reactions. If not, most likely one or more Peltier elements are malfunctioning. In this case, the experiment should be repeated with an adapted loading scheme to investigate the potentially defective Peltier element. In alternative, especially when individual wells present anomalous results, we recommend repeating the test on such well(s) after thorough cleaning. In fact, dirt interferes with the thermal contact between tubes and block, thereby altering the actual temperature profiles.
For amplification curves see page 12.

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

When using the rotor for 0.1 ml tubes:

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	T_a for 30 sec Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA) ⟶ acquiring to Cycling A (green and orange)
Gain setting	automatic (auto Gain)
Slope Correct	activated

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.

Note:

We recommend decreasing the annealing temperature of ca. 2 °C when using 0.2 ml tubes and corresponding rotor.

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 should be performed 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	T_a for 30 sec Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA)

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 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 are considered negative.

Mx3005P™

- Go to the setup menu, click on **Plate Setup**, check all positions that apply
- Click on **Collect Fluorescence Data** and check FAM™ and ROX™
- According 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	T_a for 30 sec Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA)
	data collection end
40 cycles	

- In the menu **Run Status,** select **Run** and start the cycler by pushing **Start**

Analysis of raw data:

- In the window **Analysis,** tap on **Analysis Selection/Setup** to analyze 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 automatic thresholding. Adapt the threshold line to the initial linear section of the positive controls
- Read the Ct values at **Text Report**

CFX96 Touch™

Run Setup - Protocol Tab

- Click on **File->New->Protocol** to open the Protocol Editor and create a new protocol.
- Select any step in either the graphical or text display. The selected step becomes highlighted in blue. Click on the temperature or incubation time to directly edit the value (see below).

PCR Program

	1	2	3	4	
Temperature	95.0 °C	95.0 °C	T_a for 30 sec Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA)	40 cycles	END
Incubation time	03:00 min	00:15 sec	00:30 sec		

Run Setup - Plate Tab

- Click on the **Plate Tab** to open the Plate Editor and on **Create New** 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 select the fluorophores that will be used in the run.
- Select the wells to be loaded within the plate diagram.

Run Setup – Start Run Tab

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

Data Analysis: Quantification Tab

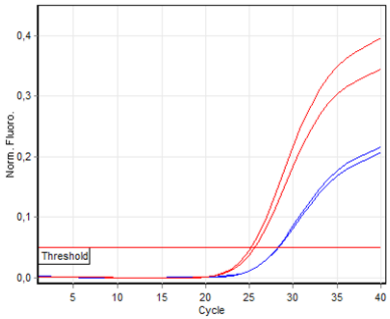
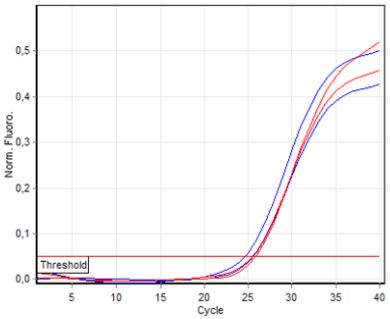
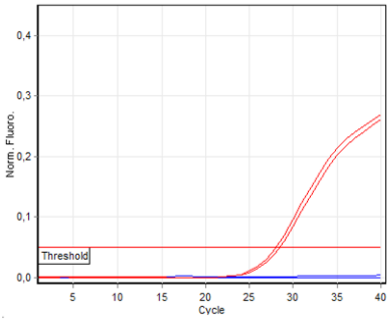
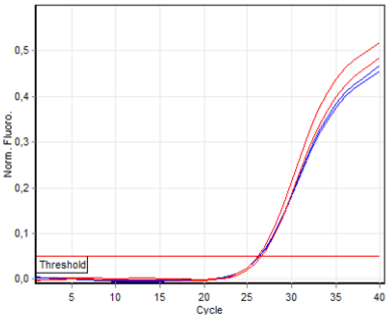
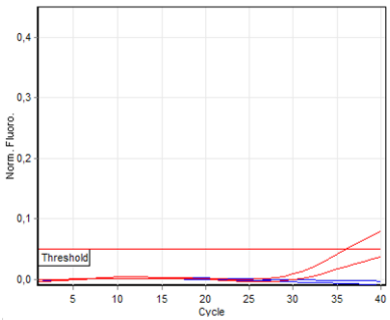
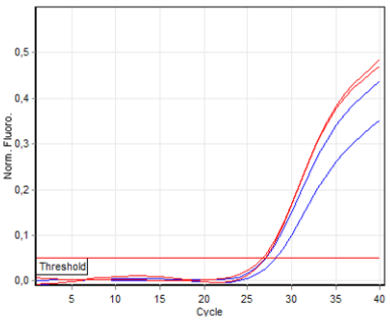
The amplification chart data in this tab displays the relative fluorescence units (RFU) collected from each well at every cycle of the run.

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

Data Analysis Settings

- The software uses two modes for quantification cycle (Cq) determination. Select **Settings** from the menu bar and in **Baseline Setting**, **Baseline Subtracted Curve Fit** and **Single Threshold** as **Cq Determination Mode**.
- In the single threshold mode, click and drag the threshold line to manually set the line position. 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.

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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Mx3005P is a trademark of Agilent Technologies. CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc.

Venor and Onar are registered trademarks and Cycler Check, Food Control, Meat ID, Vegan Control, AquaScreen, ExtractNow, PCR Clean, LabClean, WaterShield, ConviFlex, and SwabUp are trademarks of Minerva Biolabs GmbH, Germany.

Related Products

Conventional PCR Cycler Validation kits		
57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	qPCR Cycler Check™ OneStep	100 reactions
PCR Mix		
191-0025/-0100/-0250	ConviFlex™ DNAmp Mix, PCR Mix with Taq polymerase for conventional and qPCR	25/100/250 reactions
192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, RT-PCR Mix with Taq polymerase and retrotrascriptase for conventional and RT-qPCR	25/100/250 reactions
SwabUp™ Lab Monitoring Kits		
181-0010/-0050	Sample collection and DNA extraction	10/50 samples
Food and Water Assays		
36x-x025	Food Control™ qPCR	25 reactions
370-1025/-1100	Meat ID™ Halal	25/100 reactions
370-2025/-2100	Vegan Control™	25/100 reactions
34-2025/-2100/-2250	AquaScreen® Legionella pneumophila	25/100/250 reactions
33-2025/-2100/-2250	AquaScreen® Legionella species	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® Pseudomonas aeruginosa	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® Escherichia coli	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 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 reactions
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 reactions
Contamination Control Kits for qPCR		
11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions
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/-2200	DNA Decontamination Reagent, Spray bottle/refill bottles	250 ml/4 × 500 ml
15-2001	DNA Decontamination Reagent, Wipes in a dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes in refill bags	5 × 50 wipes
LabClean™		
15-4100	DNA Decontamination Reagent, bottle	1 l
WaterShield™		
15-3015/-3020/-3050	Water Disinfection Additive for incubators and water baths, 200× concentrate	15 × 10 ml/3 × 50 ml/500 ml
ZellShield™		
13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/5 × 50 ml

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