PCR Cycler Check™ Advance / OneStep

For conventional PCR block 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. Such cases are critical but can be identified by assessing the temperature accuracy of the PCR cycler. However, temperature assessment of a PCR cycler needs special and therefore expensive equipment, such as temperature sensors that measure the temperature homogeneity in a cycler block.

The PCR Cycler Check™ kit is specifically designed for verifying conventional PCR cyclers, particularly for 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

The PCR Cycler Check™ kit is based on a temperature-sensitive PCR assay to monitor an upper and lower temperature range in one run. The primer sequences in combination with a regular PCR protocol were designed to be extremely sensitive to fluctuations in temperature and thermal 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 band patterns. The cycler performance is tested with typical PCR settings to reflect most users' 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

Each kit contains all reagents required to run the PCR. The expiry date of the unopened package is marked on the package label. The kit components must be stored until use at +2 to +8 °C. Do not freeze or store the Validation Reagent after reconstitution.

Component	Quantity	
	Advance Cat. No. 57-2102	**OneStep Cat. No. 57-2103**
Validation Reagent	Validation Strips: 6 strips, 8 vials each, lyophilized, pre-loaded	Validation Tubes: 4 vials, for 25 reactions each, lyophilized, red cap
Caps	6 cap strips, domed	n.a.
Rehydration Buffer	1 vial, 1.6 ml, blue cap	2 vials, 1.6 ml each, blue cap
Marker	1 vial, 50 µl, green cap	2 vials, 50 µl each, green cap

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 PCR Cycler Check™ kit contains reagents and consumables to perform the cycler check. Additional consumables and equipment are supplied by the user:

- PCR device for 0.2 ml PCR tubes (relevant only for Cat. No. 57-2102).
- Suitable PCR reaction tubes (relevant only for Cat. No. 57-2103)
- 96-well rack for 0.2 ml PCR tubes (relevant only for Cat. No. 57-2102)
- Microcentrifuge for 8-tubes strips (relevant only for Cat. No. 57-2102) and 2 ml reaction tubes
- Vortex
- Pipettes with corresponding filter tips
- Reagents for agarose gel electrophoresis: DNA gel stain, gel running buffer
- Agarose gel electrophoresis equipment and documentation system

PRECAUTIONS

The PCR Cycler Check™ kit is for in vitro use only. The kit should be used by trained laboratory staff only. The PCR Cycler Check™ kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

ADDITIONAL NOTES

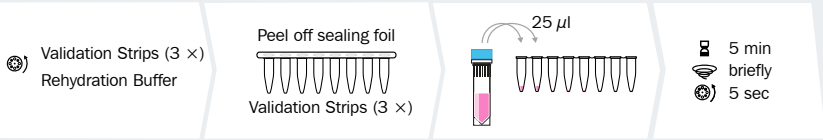
⇨ These instructions must be understood to successfully use the PCR Cycler Check™ kit. The reagents supplied should not be mixed with reagents from different batches but used as an integral unit. The reagents of the kit must not be used beyond their shelf life.

⇨ Follow the exact protocol. Any deviation may affect the test method and can affect the results.

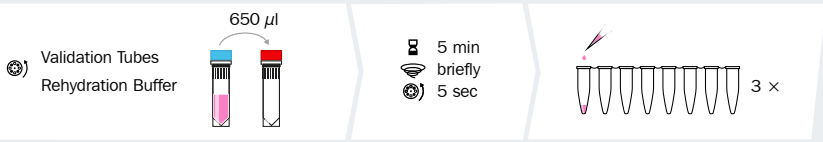
⇨ Additional control samples are not required. The kit already contains all necessary controls.

PROCEDURE – OVERVIEW

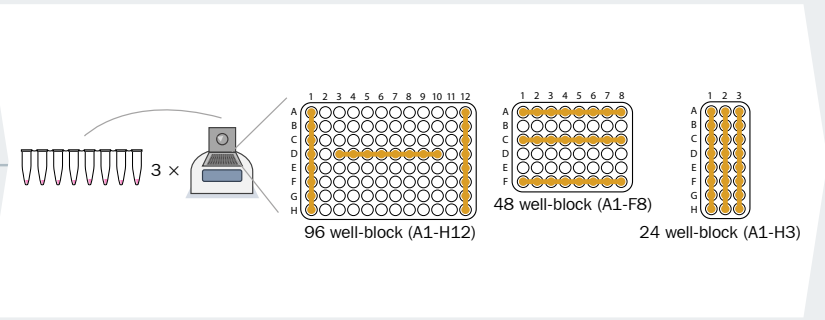
1A. Reagents preparation for Advance format (Cat. No. 57-2102)



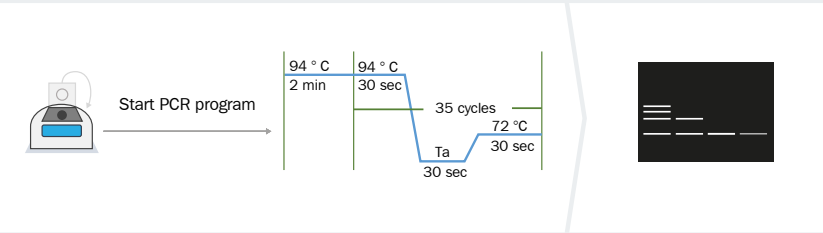
1B. Reagents preparation for OneStep format (Cat. No. 57-2103)



2. PCR amplification



3. Analysis



Validation Tubes (only for Cat. No. 57-2103)

Rehydration Buffer

Validation Strips (only for Cat. No. 57-2102)

Validation Caps (only for Cat. No. 57-2102)

Incubate

Vortex

Centrifuge

Ta Annealing Temperature

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

MB_SI_PCR_Cycler_Check_01_EN

PROCEDURE - STEP BY STEP

1A. Reagent preparation for Advance format (Cat. No. 57-2102)

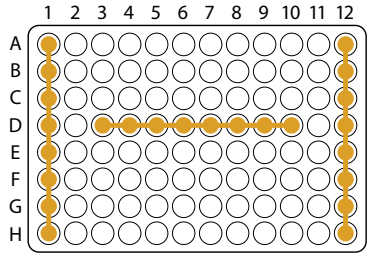
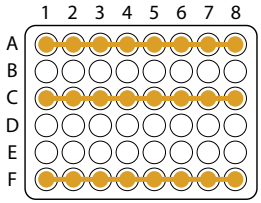
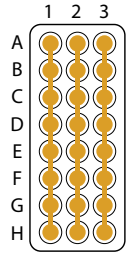
1.	Spin down the Validation Strips to collect the lyophilized material at the bottom of the tube and place the strips in a 96-well rack. Spin down the rehydration buffer.
2.	Carefully remove the protective seal from the Validation Strips.
3.	Aliquot 25 µl Rehydration Buffer into each PCR reaction tube. Close the tubes with the provided cap strips.
4.	Incubate for 5 min at room temperature.
5.	Vortex briefly and spin down for 5 sec. Proceed immediately with the PCR.

1B. Reagent preparation for OneStep format (Cat. No. 57-2103)

1.	Spin down the Validation Tubes and the Rehydration Buffer.
2.	Add 650 µl of the Rehydration Buffer (blue cap) to each Validation Tube (red cap).
3.	Incubate for 5 min at room temperature.
4.	Vortex briefly and spin down for 5 sec.
5.	Note: Proceed immediately to step 6. Do not store or freeze the rehydrated Validation Reagent. We recommend reconstituting only the Validation Tube(s) necessary to carry out the selected number of reactions (e.g. 1 vial per 24 reactions, corresponding to 1 cycler validation).
6.	Aliquot 25 µl of the rehydrated Validation Reagent into each PCR tube.
7.	Close the PCR tubes and spin down briefly. Proceed immediately with the PCR.

2. Perform the PCR cycler test

Place the PCR tubes in the cycler. We recommend the following scheme depending on the cycler block format:

96 well block	48 well block	24 well block
		

Program the cycler as follows:

Step 1 (pre-incubation):	94 °C for 2 min
Step 2 (amplification):	
Cycles	35
Denaturation	94 °C for 30 sec
Annealing	T_a for 30 sec (Annealing Temperature (Ta) is provided on the Certificate of Analysis (CoA))
Elongation	72 °C for 30 sec
Step 3:	
Hold	4 °C to 8 °C

3. Analysis

1.	Prepare a 1.5 % agarose gel including DNA stain (approx. 5 mm thick, with a 5 mm comb).
2.	Load 5 µl of each PCR reaction. Load 5 µl of the provided marker (i.e. customized DNA ladder) in one or more lanes adjacent to the samples lanes. Note: Loading buffer with dye is already included in the mixes. Thus, additional loading buffer or dye is not required.
3.	Perform the gel electrophoresis (e.g. 20 min at 100 V).
4.	Visualize the PCR results on a suitable transilluminator.

DATA INTERPRETATION

The cycler passed the test if a single band is visible (Fig. 1). The test run is valid but the cycler does not comply with the expected specifications if either no band or two bands are visible.

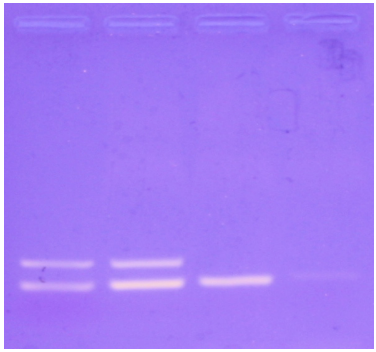
If no band is visible in any reaction, the experiment should be repeated to exclude a setup mistake. For the re-test, the annealing temperature (T_a) should be reduced by 3 °C to enhance amplification. If the re-test does not show amplification products and the cycler is already suspected to work out of specification, the device should be sent in for service.

If two bands are visible, either the setup of the test was not correct or the cycler is out of specification and should be sent in for service.

Please note, that all PCR reactions must show a uniform result. If this is not the case, most likely one or even more of the Peltier elements have a malfunction. In this case the experiment should be repeated with an adopted loading scheme.

Fragment size	Interpretation
144 bp and 210 bp	annealing temperature too low denaturation temperature ok
144 bp	Cycler test passed successfully
no bands	annealing temperature too high (s. explanation above) or/and denaturation temperature failure

Fig. 1: Gel figure showing results obtained at different annealing temperatures
A: Marker
B: Temperature too low
C: Temperature correct
D: Temperature too high



APPENDIX

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 from of the use, the results of use, or the inability to use this product.

Trademarks

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Related Products

qPCR Cycler Validation/Qualification		
57-2201	qPCR Cycler Check™	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, 200x concentrate	15 x 10 ml/3 x 50 ml/500 ml
ZellShield™		
13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/5 x 50 ml

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