PCR Cycler Check™ Advance / OneStep

For conventional PCR block 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 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Advance</strong></td>
</tr>
<tr>
<td></td>
<td>Cat. No. 57-2102</td>
</tr>
<tr>
<td>Validation Reagent</td>
<td>Validation Strips: 6 strips, 8 vials each, lyophilized, pre-loaded</td>
</tr>
<tr>
<td>Caps</td>
<td>6 cap strips, domed</td>
</tr>
<tr>
<td>Rehydration Buffer</td>
<td>1 vial (1.6 ml)</td>
</tr>
<tr>
<td>Marker</td>
<td>1 vial (50 µl)</td>
</tr>
</tbody>
</table>

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
- Vortexer
- 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

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:

<table>
<thead>
<tr>
<th>96 well block</th>
<th>48 well block</th>
<th>24 well block</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2 3 4 5 6 7 8</td>
<td>1 2 3 4 5 6 7 8</td>
<td>1 2 3</td>
</tr>
<tr>
<td>A B C D E F G H</td>
<td>A B C D E F G H</td>
<td>A B C D E F G H</td>
</tr>
</tbody>
</table>

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 (\( T_a \)) 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).

   Load 5 \( \mu l \) of each PCR reaction. Load 5 \( \mu l \) 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.

2. Perform the gel electrophoresis (e.g. 20 min at 100 V).

3. Visualize the PCR results on a suitable transilluminator.
RESULT 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<sub>a</sub>) 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.

<table>
<thead>
<tr>
<th>Fragment size</th>
<th>Interpretation</th>
</tr>
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</table>
| 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 the use, the results of use, or the inability to use this product.

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**Related Products**

**qPCR Cycler Check™**
57-2201 qPCR Cycler Validation 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 reactions
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 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

**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/-2500 DNA Decontamination Reagent, Spray bottle/refill bottles 250 ml/4 × 500 ml/5 l
15-2001 DNA Decontamination Reagent, Wipes in a dispenser box 120 wipes
15-2002 DNA Decontamination Reagent, Wipes in refill bags 5 × 120 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