

Instructions for use  
**BAG Cyclor Check**

Test kit for validation of temperature uniformity in thermal cyclers

**ready for use, pre-aliquoted**

**REF 7104 (10 tests)**

**REF 71044 (4 tests)**

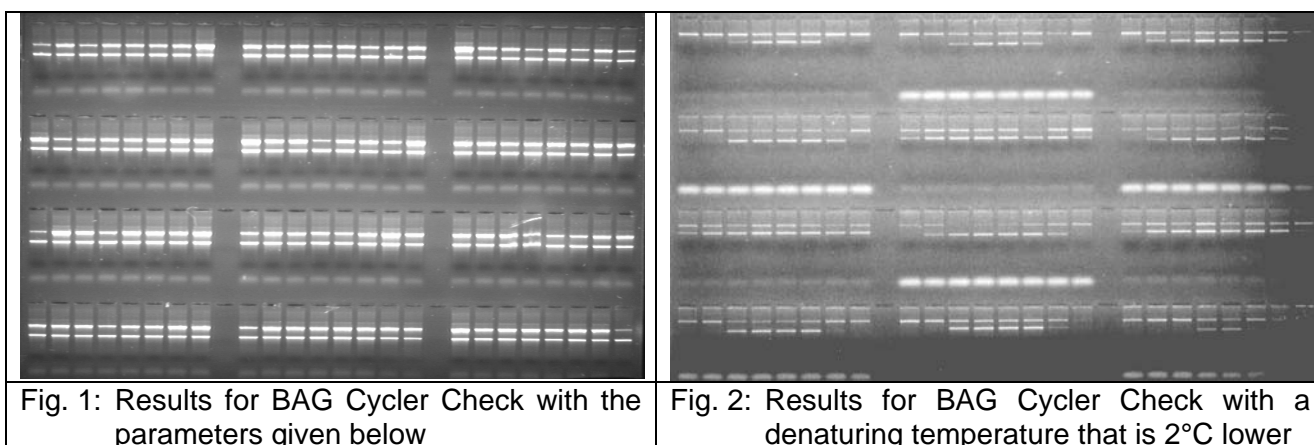
**Contents**

|   |   |
|---|---|
| 1. Product description .....                  | 2 |
| 2. Material .....                             | 3 |
| 2.1 Contents of the BAG Cyclor Check .....    | 3 |
| 2.2 Supplementary material.....               | 3 |
| 2.3 Storage and stability .....               | 4 |
| 3. Test procedure.....                        | 4 |
| 3.1 Amplification .....                       | 4 |
| 3.2 Gel electrophoresis .....                 | 5 |
| 3.3 Documentation and interpretation .....    | 6 |
| 4. Warnings and precautions .....             | 6 |
| 5. Troubleshooting .....                      | 7 |
| 6. Explanation of symbols used on labels..... | 7 |

## 1. Product description

The BAG Cycluser Check is a fast and easy method for validation of temperature uniformity in thermal cyclers. Especially in diagnostics of nucleic acids it is essential to ensure the dependability of the thermal cycler in use. Therefore, such a test should be done regularly. The BAG Cycluser Check consists of one reaction mix which is tested in all 96 positions of a thermal cycler block. The reaction mix contains one primer pair for validation of the denaturing temperature (540 bp) and another primer pair for validation of the annealing temperature (1040 bp). With these two primer pairs it can be tested if the denaturing temperature is lower or the annealing temperature is higher than it should be.

If the temperatures are correct and the temperature profile is uniform, there should be two bands for all 96 positions of the block (Fig 1). Differing temperatures result in the loss of bands in single positions or in all positions (Fig. 2).



Since the test mix in the BAG Cycluser Check fits the conditions of the BAG HISTO TYPE kits, it is very well suited to optimize the PCR parameters on an individual thermal cycler. This may be necessary because thermal cyclers may be adjusted quite differently.

## **2. Material**

### **2.1 Contents of the BAG Cycler Check kit**

- ◆ 4 or 10 BAG Cycler Check plates sufficient for 4 or 10 tests. The dried reaction mixtures consists of two primer pairs and nucleotides.
- ◆ 1 or 2 x 1,1 ml 10 x PCR-buffer
- ◆ 2 or 5 x 210 µl control DNA (60 ng/µl)
- ◆ 4 or 10 x PCR foils sufficient for 4 or 10 tests
- ◆ instructions for use and test protocol

### **2.2 Supplementary material**

- ◆ Taq Polymerase (5 U/µl)
- ◆ piston pipettes (0,5-250 µl)
- ◆ sterile tips with integrated filter
- ◆ DNA Cycler (e.g. PTC 200 with a heated and adjusted cover, MJ Research/Biozym)

### **Devices and material for gel electrophoresis**

- ◆ DNA agarose
- ◆ 0.5 x TBE buffer (45mM of Tris, 45 mM of boric acid, 0,5 mM of EDTA)
- ◆ Ethidium bromide (EtBr)
- ◆ submarine electrophoresis unit
- ◆ power supply (200-300 V, 200 mA)

### **Devices for interpretation and documentation**

- ◆ UV source (220-310 nm)
- ◆ camera (e.g. Polaroid system) with films (Polaroid type 667)

## 2.3. Storage and stability

The kit is delivered unfrozen. Store all reagents at -20°C to - 80°C in the dark. Stability as indicated on the outer label refers to all reagents contained in the kit. Shortly before using, thaw the 10 x PCR-buffer.

## 3. Test procedure

### 3.1 Amplification

- Pipet the Master-Mix consisting of 10 x PCR-buffer, DNA solution, Taq-Polymerase and Aqua dest and vortex thoroughly.

**104 µl control DNA (60 ng/µl)**

**824 µl distilled water**

**104 µl 10xPCR buffer**

**8,3 µl Taq polymerase (5 U/µl)**

- Add **10 µl** of this mixture to the pre-dropped reaction mixtures. Tightly close the tubes with the respective foil. With cyclers with an adjustable lid re-usable PCR mats can also be used. Slightly shake the plate downwards to dissolve the blue pellet at the bottom of the plate. All PCR solution should settle on the bottom. Overlaying of the reaction mixtures with mineral oil is **not** required if a heated and adjusted lid is used!
- Place the reaction tubes into the thermal cycler and tighten the lid. The reaction tubes should be tightly fit in the block. Start the PCR program. The position of the plate in the cycler block (A1 at the top and at the left side) is important to be able to reassign the reactions to the positions in the cycler.

### Amplification parameters:

| Programme-Step      | Temp. | Time   | No. of Cycles |
|---------------------|-------|--------|---------------|
| First Denaturation  | 96°C  | 5 Min  | 1 Cycle       |
| Denaturation        | 96°C  | 20 Sec | 5 Cycles      |
| Annealing+Extension | 68°C  | 1 Min  |               |
| Denaturation        | 96°C  | 20 Sec | 10 Cycles     |
| Annealing           | 64°C  | 50 Sec |               |
| Extension           | 72°C  | 45 Sec |               |
| Denaturation        | 96°C  | 20 Sec | 15 Cycles     |
| Annealing           | 61°C  | 50 Sec |               |
| Extension           | 72°C  | 45 Sec |               |
| Final Extension     | 72°C  | 5 Min  | 1 Cycle       |

### Cycler types:

PTC 100 / 200  
(MJ Research/  
Biozym)  
and  
GeneAmp PCR-  
System 9600 / 9700  
(Perkin Elmer)

### 3.2 Gel electrophoresis

Separation of the amplification products is done by electrophoresis via a (horizontal) agarose gel. As electrophoresis buffer, 0.5 x TBE (45 mM of tris, 45 mM of boric acid, 0.5 mM of EDTA) buffer is recommended. The gel concentration should be 2.0 - 2.5% of agarose. Allow the gel to polymerize at least 30 minutes before sample loading. After amplification has been finished, take the samples out of the thermal cycler and load the complete reaction mixtures carefully in each slot of the gel. In addition, apply 10 µl of the DNA length standard for size comparison. Electrophoretic separation is done at 10-12 V/cm (with 20 cm distance between the electrodes approx. 200-240 V), for 20-40 min. After the run has been completed, the complete gel is stained in an ethidiumbromide (EtBr) solution (approx. 0.5 µg/ml of EtBr in H<sub>2</sub>O or TBE buffer for 30-45 min.). As alternative, EtBr (0.5 µg/ml) can also be added to the electrophoresis buffer or the agarose gel. If required, excess of EtBr can be removed by soaking the gel in H<sub>2</sub>O or 0.5 x TBE buffer for 20-30 minutes.

### **3.3 Documentation and interpretation**

For documentation, visualize the PCR amplification using an UV transilluminator (220-310 nm) and photograph it with a suitable camera, film and filters (e.g. polaroid, film type 667). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background (approximates aperture 11, exposure time 1 second).

Possible results:

- If the temperatures in the thermal cycler are correct all over the block there should be two bands in all positions (540 bp + 1040 bp).
- If the denaturing temperature is too low the smaller band will be missing in some or in all positions.
- If the annealing temperature is too high first the bigger than also the lower band will be missing in some or in all positions.
- If the annealing temperature is too low there may be unspecific bands.

If the PCR result does not match the requirements, the temperatures should be checked with an electronic measuring device and if necessary the customer service for the instrument should be called.





## **4. Warnings and Precautions**

Ethidiumbromide is a powerful mutagen. Wear gloves when handling gels or solutions containing EtBr! Note the instructions for use and warnings and precautions of the manufacturer! The transilluminator radiates very short-wave UV light which may cause burns of the skin and the retina. Use a UV face safety mask!

## 5. Troubleshooting

| Problem   | Possible Reason                                       | Solution  |
|---|---|---|
| No amplification,<br>length standard visible                  | • enzyme is missing or concentration too low          | • repeat typing, alter enzyme concentration                     |
|   | • wrong amplification parameters                      | • optimize the amplification parameters, check cycler           |
| No or only very weak bands visible, length standard invisible | EtBr staining too weak                                | repeat staining   |
| Gel background<br>Shines too bright                           | staining was too long,<br>EtBr concentration too high | soak gel in H <sub>2</sub> O or TBE<br>lower EtBr concentration |
| Blurred band  | Electrophoresis buffer too hot                        | Lower the voltage   |
|   | Wrong electrophoresis buffer                          | Use 0,5x TBE buffer   |

## 6. Explanation of symbols used on labels

|   |                              |
|---|------------------------------|
|  | Storage temperature          |
|  | Batch code                   |
|  | Use by                       |
| REF   | Catalogue number             |
|  | Consult instructions for use |

### ↑ Notice to Purchaser: Disclaimer of License

This product is optimized for use in the Polymerase Chain Reaction ("PCR") Process which is covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche Ltd ("Roche"). No license under these patents to use the PCR Process is conveyed expressly or by implication to the purchaser by the purchase of this product. Further information on purchasing licenses to practice the PCR Process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California, 94501.



BAG Health Care GmbH  
Amtsgerichtsstraße 1-5  
35423 Lich/Germany

Tel.: +49 (0) 6404 / 925-0    www.bag-healthcare.com  
Fax: +49 (0) 6404 / 925-250    info@bag-healthcare.com

Auftragsannahme/Ordering:  
Tel.: +49 (0) 6404 / 925-450  
Fax: +49 (0) 6404 / 925-460  
verkauf@bag-healthcare.com

Customer Service:  
Tel.: +49 (0) 6404 / 925-125  
Fax: +49 (0) 6404 / 925-421  
service@bag-healthcare.com