EN

Instructions for use

CYCLER CHECK

Electronic instructions for use see www.bag-diagnostics.com

Test kit for validation of temperature uniformity in thermal cyclers

ready to use, prealiquoted

REF 7104 (10 tests)

REF 71044 (4 tests)

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1. Product description

The CYCLER 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 CYCLER 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.

Just in case that the denaturation temperature (96°C) cannot be achieved, false negative results may occur, especially in presence of GC rich sequences, which prone to secondary structures.

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).

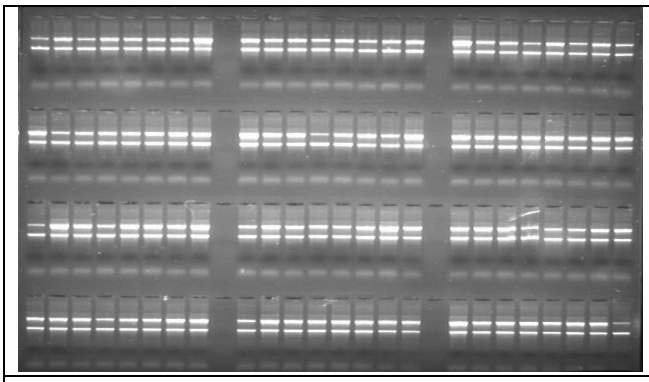

Fig. 1: Results for CYCLER CHECK with the parameters given in 3.1

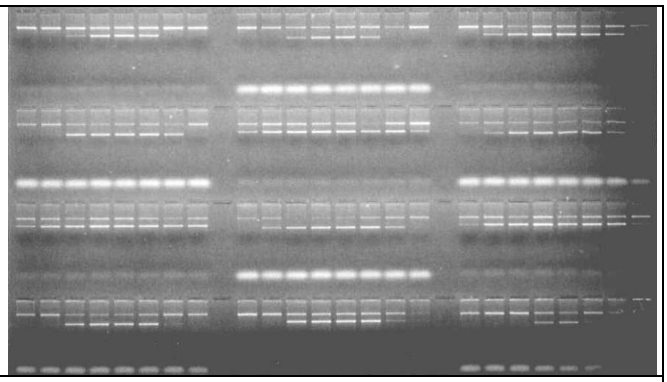

Fig. 2: Results for CYCLER CHECK with a denaturing temperature that is 2°C lower

Since the test mix in the CYCLER 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 CYCLER CHECK kit

- 4 or 10 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

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2.2 Supplementary material

- Taq Polymerase (5 U/µl), (Happy Taq REF 70976 or another Taq Polymerase, validated with the CYCLER CHECK kit by the user)
  Don't use a Hot-start Taq Polymerase please!
- piston pipettes (0.5-250 µl)
- sterile tips with integrated filter
- DNA Cycler (list of the validated cyclers please see chapter 3.1)

Devices and material for gel electrophoresis

- DNA agarose
- 0.5 x TBE buffer (45 mM 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) or video system with thermal paper (e.g. Typ KP65HM-CE)

2.3. Storage and stability

The kit is delivered at ambient temperature. Store all reagents at **≤ -20°C** in the dark in a temperature monitored device after receiving. The expiry date is indicated on the label of each reagent and is also valid for opened reagents. The expiry date indicated on the outer label refers to the reagent with the shortest stability contained in the kit. Shortly before using, thaw the 10 x PCR-buffer.

3. Test procedure

The evaluation and quality control were done with the Happy Taq (REF 70976).

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 10x PCR buffer
8,3 µl Taq polymerase (5 U/µl)

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- Add **10 µl** of this mixture to the pre-dropped reaction mixtures. Tightly close the tubes with the respective foil. Ensure that you do not touch the inner side of the caps and the upper edges of the tubes with the fingers to avoid contamination. If cyclers with tightly closable lid are used, it is also possible to use reusable PCR mats. Slightly shake the plate to dissolve the pellet at the bottom of the plate. All PCR solution should settle on the bottom. If necessary the plate should be briefly spun down. Overlaying of the reaction mixtures with mineral oil is **not** required if a heated and adjusted lid is used!

- Place the reaction tubes firmly into the thermal cycler and tighten the lid. 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

Validated Cycler types:
PTC 100 / 200 / C1000 (MJ Research/ BioRad), GeneAmp PCR-System 9600 / 9700 (use heating rate of 9600 please), Veriti (ABI), Mastercycler epGradient S (use “simulate Mastercycler gradient” function please) (Eppendorf)
Tprofessional (Biometra)

Please don’t use an aluminium heating block (e.g. GeneAmp PCR-System 9600 / 9700)!

When using thermal cyclers with a very fast heating and cooling rate, it is recommended to use a reduced ramp rate (~ 2.5°C/sec).

The quality control tests were done on a PTC-200 resp. C1000 (MJ Research / BioRad), 9700 (ABI), Mastercycler epGradient S (Eppendorf) and Tprofessional (Biometra).

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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 minutes. 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_2O or TBE buffer for 30 - 45 minutes). 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_2O 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 or video system, thermal paper KP65HM-CE). Choose exposure time and aperture such that the bands are drawn sharp and stand out against the dark background.

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 **540 bp band** will be missing in some or in all positions.
- If the annealing temperature is too high first the **1040 bp band** than also the **540 bp 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.

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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!

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

A declaration on Material Safety Data Sheets (MSDS) is available to download at www.bag-diagnostics.com .

5. Troubleshooting

Problem	Possible Reason	Solution
No amplification, length standard visible	enzyme is missing	repeat typing
	enzyme concentration too low	alter enzyme concentration
	wrong amplification parameters	optimize the amplification parameters, check cycler
No or only very weak bands visible, length standard invisible	staining too weak	repeat staining
Gel background shines too bright	staining was too long	soak gel in H_2O or TBE
	staining solution concentration too high	lower staining solution concentration
Blurred band	electrophoresis buffer too hot or used up	lower the voltage, use fresh 0.5 x TBE buffer
	wrong electrophoresis buffer	use fresh 0.5 x TBE buffer
	polymerisation of the gel not well	use completely polymerised gel

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6. Explanation of symbols used on labels

	Storage temperature / Lower limit of temperature
	Use by
	Manufacturer
	Consult instructions for use
	Sufficient for n tests
CONT	Content, contains
CONTROL DNA	Control DNA
CYCLER CHECKING	Intended Purpose: Validation of temperature uniformity in thermal cyclers
IFU	Instructions for use
LOT	Batch code
PCRBUF 10x	PCR buffer, 10x concentrated
PCRFOIL	PCR foil
PCRPLATE	PCR plates
REACTIONMIX	Reaction mixes
REF	Catalogue number
RTU	Ready to use

Instructions for use in other languages see:
http://www.bag-diagnostics.com or contact us directly at info@bag-diagnostics.com
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