PCR Cycler Validation Kit

Product No. A9742

Description

False negative or non-specific PCR results are highly critical and might be caused by a defect heating element (peltier element) of the PCR cycler. Control of the reaction temperature is therefore essential. The PCR Cycler Validation Kit provides a temperature sensitive PCR reaction to monitor inconsistencies in the temperature range of the thermal cycler. The primer sequences and the PCR protocol were designed to detect fluctuations >2°C by altered amplification, indicated by different band pattern. Furthermore, the pre-adjusted target concentrations are only amplified at high PCR efficiencies as an additional indicator for accurate temperature control of the thermal cycler. This reference setup suits to reliably evaluate all relevant parameters of the process: temperature homogeneity, precision of the temperature control, and the timing can be verified. The PCR results are visualized by gel electrophoresis at the endpoint of the PCR reaction.

PCR Cycler Validation Kit A9742,0002 contains kit components for the run of 2 cycler validation processes on one cycler or one validation on two individual block heated cyclers equipped with cavities for 0.2 ml PCR tubes.
The Kit is applicable with any block PCR cycler in research or industrial quality assurance lab in order to fulfill legal requirements for the reliability testing of instruments used for analysis (ISO 17025, EN 45001, ISO 13485, GLP, GMP).

Kit Components

Label Information	Quantity	Cap Color
Qualification Tubes A9742,0002A	6 strips with 8 tubes each, containing lyophilized primer sets, polymerase and nucleotides.	-
Tube lids A9742,0002B	6 x 8 strips, domed	-
DNA size marker A9742,0002C	1 x 50 µl	green
Rehydration Buffer A9742,0002D	1 x 1.6 ml	blue

The kit components are stored until use at +2 to +8 °C.

Shelf Life and Storage
Components are maintainable at 2 to 8°C for at least 6 months.
After reconstitution: For best results the reagents should be used immediately after rehydration.
(If it however becomes necessary that reagents need to be stored after reconstitution, please store at -20°C. Avoid repeated freeze-thaw cycles.)

Required consumables and devices

- PCR reaction tubes
- Reagents for agarose gel electrophoresis
- Required lab devices
- Tube centrifuge
- Pipetting equipment
- PCR cycler
- Agarose gel electrophoresis and documentation system

For use in research and quality control. This kit should be used only by trained persons. This kit does not contain hazardous substances and may be disposed of according to local regulations.

Protocol

The validation should be precisely performed as described for a successful use of the PCR Cycler Validation Kit. The kit components should not be mixed with reagents from different lots and beyond its shelf life.
Please note that any deviation from the test method can affect the results. The use of control samples is not required as the PCR set contains a setup check.

1. Rehydration of the reagents

Please note that after reconstitution, the reagents should be used immediately!

1. Centrifuge or tip the Qualification Tubes on the table to accumulate the lyophilized material at the bottom of the tube.
2. Peel off protective film from Qualification Tubes.
3. Aliquot 25 µl of the Rehydration Buffer into each PCR reaction tube. Close the tube tightly.
4. Incubate 5 min at room temperature
5. Vortex briefly and spin for 5 sec

2. Loading the Qualification Tubes

The loading scheme depends on the block format of the PCR cycler (see Figure 1). The following schemes are recommended for regular testing. If particular Peltier elements, segments of the block or cavities are already subject of suspicion, the strips or even individual tubes can be placed flexible within the block.

Example 1: Regular testing covering all Peltier elements. The number of Peltier elements commonly varies between 1-4, depending on the cycler type and manufacturer.

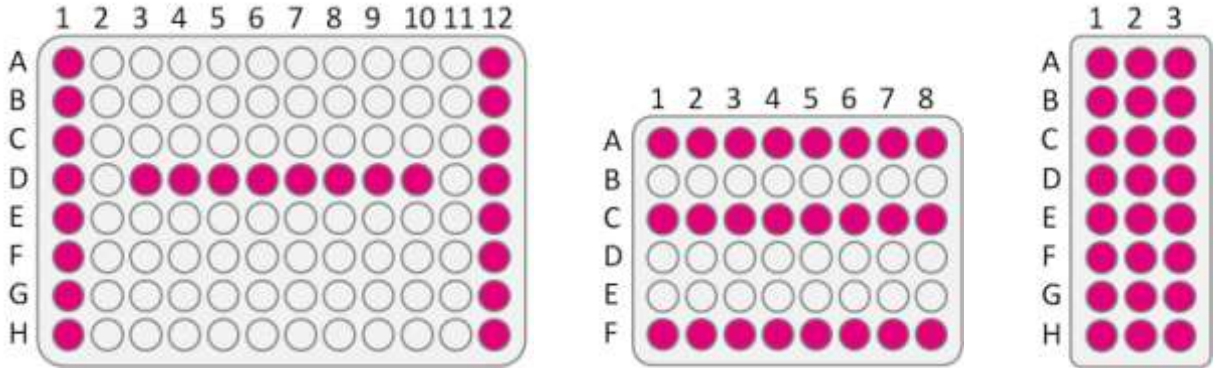
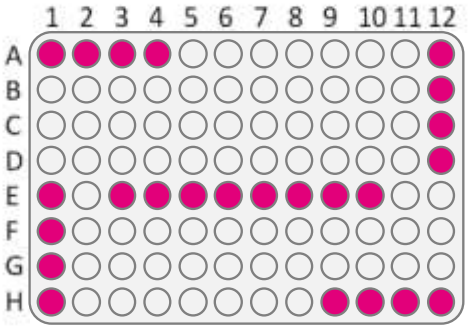
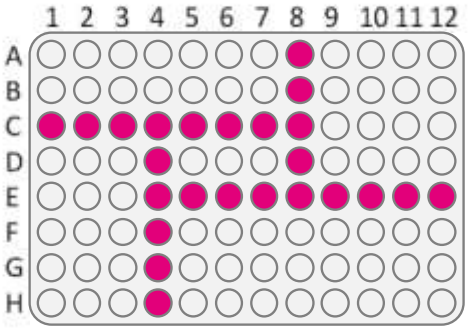


Figure 1: Recommended test schemes for different heating blocks: 96 well block, 48 well block, 24 well block. The colored wells indicate the positions of the test strips.

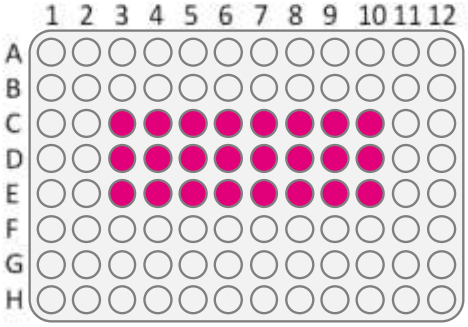
Example 2: Testing of critical positions such as corner and border positions. This testing is recommended if the whole cycler block is used on a regular base:



Example 3: Closing pressure of the cycler lid. Especially older cyclers can suffer from a weakening of the closing device resulting in a decreased contact between PCR tube and well surface in the center positions of the heating block. To visualize such a weakening we suggest the following positioning of the test strips:



Example 4: For users that run only a few reactions at the same time testing of the commonly used wells is recommended. Single wells can be faulty due to contaminations resulting from inadequate handling or leaky PCR tubes. Salty crusts on the surface of a well might reduce the interface between tube and well leading to reduced heating.



3. Starting the reaction

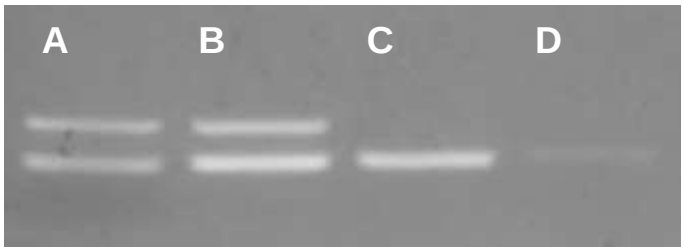
Edit and start the following cycler program. The programming process of your cycler is explained in the manual of the instrument:

1 cycle	94 °C	for 2 min
35 cycles	94 °C	for 30 sec
	67 °C	for 30 sec
	72 °C	for 30 sec
cool down 4 °C to 8 °C		

4. Visualization and evaluation of results

Prepare a 1.5% standard agarose gel, approx. 5 mm thick, with a 5 mm-comb. Load 5 µl of each PCR reaction and of the marker provided with the kit in each lane. No loading buffer and running dye are required. Stop electrophoresis after 2 cm run distance (time depends on the electrophoresis conditions used, e.g. run for 20 minutes at 100 V).

The thermo cycler's setup is correct if one band is visible on the gel (see Figure 2 and table below).
If the results are valid but the cycler does not comply with the expected specifications either no band or two bands are visible:



Lane A: DNA Marker (provided)
Lane B: Temperature too low.
Lane C: Temperature correct.
Lane D: Temperature too high.

Figure 2: Agarose gel with all possible results

If none of the bands are visible in all reactions the experiment should be repeated to exclude a setup mistake. For the re-run the annealing temperature should be reduced from 67 to 64 °C to provoke amplification. If the re-run does not show amplification products and if the cycler is already under suspect for abnormal routine PCR results the device should by send in for service.
If two bands are visible either the setup of the test was not ok or the thermal cycler shows a fatal error.
Please note, that all PCR reactions should show a uniform result. If not, most likely one or even more of the Peltier elements show a malfunction. In this case the experiment should be repeated with an adopted loading scheme.

Result	Interpretation
2 bands (144 bp and 210 bp)	Lower temperature range failure Annealing temperature failure Denaturation temperature ok
1 band (144 bp)	Setup check ok
no band	Higher temperature range failure Annealing temperature failure Denaturation temperature failure