



Instrument performance verification – Validation of PCR cyclers

Instrument models covered:

PCR cyclers by all manufacturers

PCR Cycler instrument verification consists of:

- **Instrument functionality check**
 - Low voltage internal power supply check
 - Display and keyboard controls functionality
- **PCR cycler temperature validation**
 - Temperature Accuracy Validation – Two-point validation at 85°C and 45°C
 - Temperature Transition Validation – In the range from 95°C to 35°C (or to 4°C according to the PCR cycler type)
 - Validation of the standard temperature cycle durability - Validation of the duration of 6 consecutive standard cycles (95°C and 55°C for 30 seconds)
 - Validation of block temperature uniformity - Validation of temperatures 95°C and 60°C (or other values depending on the cycler type) for 8 different sample block positions
- **Documentation**

After the device validation, the following documentation is provided to the customer:

 - PCR cycler validation protocol (for each block separately)
 - Calibration protocol of the measuring assembly (Copy)
 - Certification of the service engineer training for PCR cycler validation (Copy)
 - Certification of the service engineer technical expertise in electrical engineering (Copy)

METHODOLOGY OF PCR-qPCR CYCLER TEMPERATURE VALIDATION

We use following calibrated measuring sets:

- FLUKE THERMOMETER digital display, type CNX t3000, measuring range -220 to +1150°C, accuracy $\pm 0.2^\circ\text{C}$ (for the entire range of measurement). The GREISINGER temperature probe, type GTF101-5, is used in the measuring set. The FLUKE THERMOMETER CNX t3000 digital display is calibrated with the GREISINGER GTF101-5 temperature probe as a measuring assembly in the temperature range from 0°C to 95°C.
- ALPHA TECHNICS THERMOMETER digital display, type 4690, measuring range 0 to +110°C, accuracy $\pm 0.025^\circ\text{C}$ (for the entire range of measurement). ALPHA TECHNICS temperature probes are used in the measuring set, probe types: GEMINI PROBE MODULE 9-CH 0,2mL (accuracy ch.1-8 $\pm 0.100^\circ\text{C}$ and ch. 9 $\pm 1.000^\circ\text{C}$ for the entire range of measurement), PROBE MODULE 9-CH 0,1ml (accuracy ch.1-8 $\pm 0.200^\circ\text{C}$ and ch. 9 $\pm 1.000^\circ\text{C}$ for the entire range of measurement), SEDONA PROBE MODULE 9-CH 0,2mL (accuracy ch.1-8 $\pm 0.100^\circ\text{C}$ and ch. 9 $\pm 1.000^\circ\text{C}$ for the entire range of measurement). The ALPHA TECHNICS THERMOMETER 4690 and temperature probes are calibrated as a measuring assembly in the temperature range from 0°C to 110°C.

Temperature measurement is performed:

- Using the FLUKE THERMOMETER CNX t3000 digital display with the GREISINGER GTF101-5 temperature probe immersed in 50 μL of mineral oil (for 200 μL well cyclers) or 20 μL of mineral oil (for 100 μL well cyclers) in a commonly used tube, the type of which depends on the type of the PCR cycler used. This ensures that all measured values are measured as the real sample temperature, not the sample block temperature. Values measured are recorded in the validation protocol.
- Using the ALPHA TECHNICS THERMOMETER type 4690 - GEMINI PROBE and PROBE MODULE have nine channels for sample wells having volume of 200 or 100 μL . SEDONA PROBE MODULE has nine channels for PROFLEX blocks, sample well volume 200 μL . For all probe types the measurement is made simultaneously in 8 block wells and the 9th channel is used to measure the heated lid temperature at the same time. Values measured are recorded in the validation protocol.
- At the customer's request, all temperatures can be recorded and subsequently their graphical record can be attached to the validation protocol. Temperature recording is performed at 1 second interval for the entire measurement time.