Calibration Certificate Optical

On site Calibration

Certificate nr: **DO**A75012402-BY73 (rev.1)
Driftcon® Service License 2

Applicant

Institute / Client	Cyclertest
Department	training and support
Address	Rotcherweg 61
Zip / City	6374XE Ladngaarf
Country	Netherlands

On site location

Contact	Support
Address	Rotschereweg 61
Room	demo room 1 floor
Zip / City	6374 XW Landgraaf
Country	Netherlands
Phone	+31455338733
E-mail	

Tested instrument (DUT)

Manufacturer	Bio-Rad Laboratories
Instrument	CFX96 Opus Deepwell
Instrument serial number	CT011239
Block type	96-Deep Well
Block position	Center
Block serial number	RD031799
Optical unit serial number	790BR01182
Internal reference	cycler_07
Software version	3.1.1517.0823

Calibration method

The optical calibration is performed conform ISO 20836:2021 guidelines.
15 LED's (light emission diode) and one temperature sensor are position on top of the calibration unit. The LED emit light at prescribed points in the sample block of the real-time thermal cycler. The emission intensity of light is independent of the measured temperature (Cq determination).
In addition, 19 temperature sensors measure the temperature whereas 15 of them are at the same LED prescribed points in the sample block of the qPCR instrument and are monitored through an ITS-90 traceable measurement system. The sensors are positioned at the bottom of the measurement well. Measurements are made on all temperature sensors simultaneously at 4 Hz. This physical patented Optical calibration method (CYCLERtest BV) is at the moment not fully traceability to SI however offers a substantially higher level of traceability than other methods. (conform ISO 20836:2021)
Traceability SI unit: W/sr (radiant intensity)

Thermal cycler protocol

Pre-heat
Step 1 : 30 °C for 60 seconds
Step 2 : 95 °C for 60 seconds
Step 3 : 30 °C for 60 seconds

Temperature Calibration
Step 1 : 30 °C for 60 seconds
Step 2 : 95 °C for 60 seconds
Step 3 : 30 °C for 60 seconds
Step 4 : 90 °C for 60 seconds
Step 5 : 50 °C for 60 seconds
Step 6 : 70 °C for 60 seconds
Step 7 : 60 °C for 60 seconds
Step 8 : 30 °C for 60 seconds

Optical calibration – amplification curve
Step 1 : 80 °C for 1 second
Step 2 : 60 °C for 30 seconds including detection
Repeat 24 times

Optical calibration system protocol

Pre-heat
No light emitted

Temperature Calibration
No light emitted

Optical calibration – amplification curve
Light intensity temperature independent
Light intensity gradually increased from 20% to 100% cycle depending

Non-conformities in calibration method or environmental conditions
There were no non-conformities reported.

Environmental conditions (average)

Ambient temperature	N/A
Relative humidity	N/A

Notes
Baseline Threshold Settings: Auto calculated (34.02 from DUT)

Results
The calibration results are given on following pages of this certificate.

Calibration system uncertainty
The reported uncertainty (*U*) is based on a standard uncertainty multiplied by a coverage factor of *k=2*, which provides a confidence level of approximately 95%. The standard uncertainty has been determined in accordance with EA-4/02M.

Calibration date
15-Jun-2026

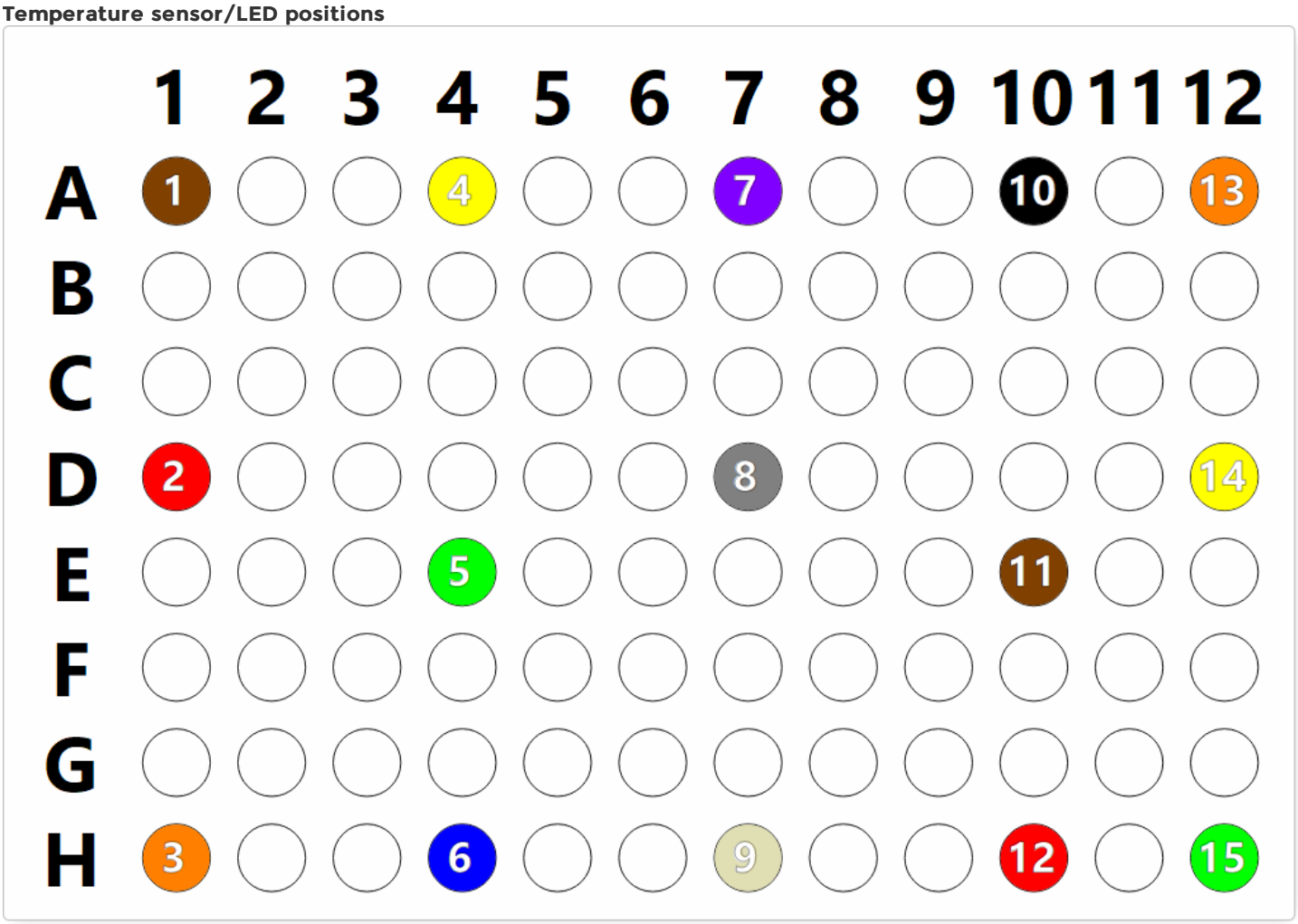
Due date
June 2028

Report date
19-Jun-2026 08:54

Approved signatory

Mr. Tom Hendrikx
CEO

Temperature sensor/LED positions



Sensor 1 in Well (A1)
Sensor 2 in Well (D1)
Sensor 3 in Well (H1)
Sensor 4 in Well (A4)
Sensor 5 in Well (E4)
Sensor 6 in Well (H4)
Sensor 7 in Well (A7)
Sensor 8 in Well (D7)
Sensor 9 in Well (H7)
Sensor 10 in Well (A10)
Sensor 11 in Well (E10)
Sensor 12 in Well (H10)
Sensor 13 in Well (A12)
Sensor 14 in Well (D12)
Sensor 15 in Well (H12)

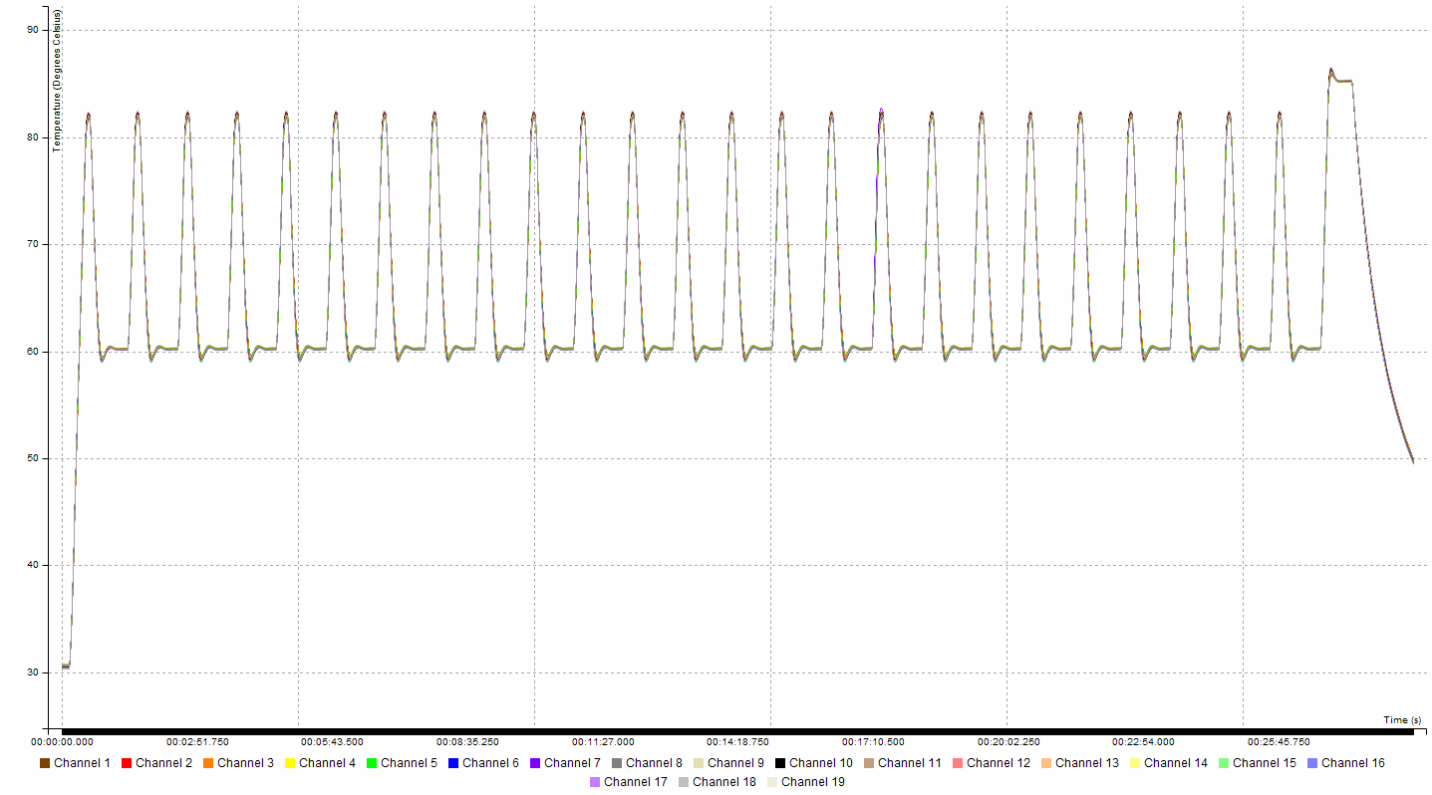
Heated lid

Heated lid sensor on top surface.

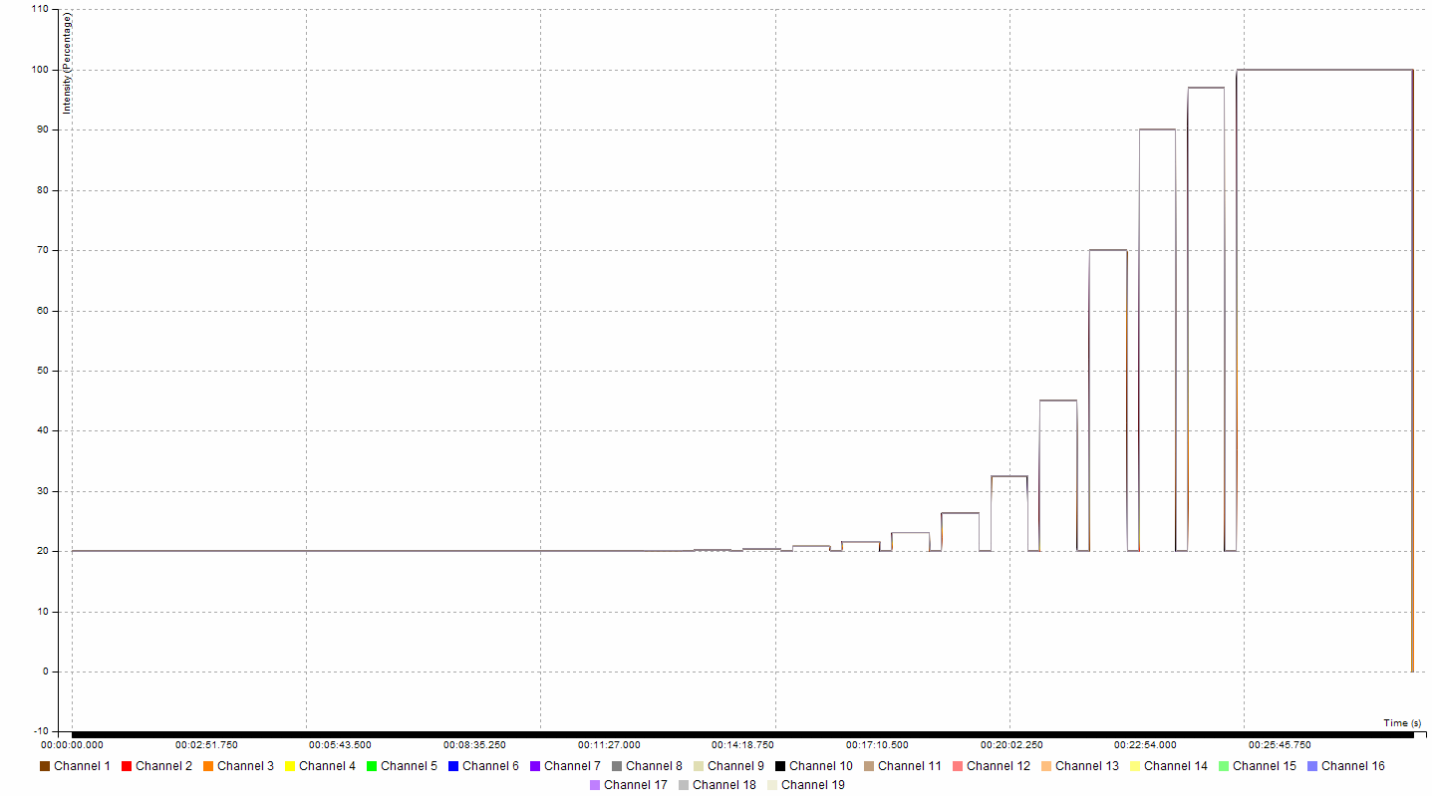
Environmental conditions

	Ambient temperature ($t_{ambient}$/ °C)	Uncertainty ($U_{ambient}$/ °C)	Limits ($t_{ambient}$/ °C)	Relative humidity (RH/%rh)	Uncertainty (U_{rh}/%rh)	Limits (RH/%rh)
Maximum	N/A	1.0	27.0	N/A	10.0	80.0
Minimum	N/A	1.0	15.0	N/A	10.0	20.0

Temperature chart of thermal cycler (measured)



Light intensity chart of optical system (emitted)



Protocol run time

0 h: 28 m: 36 s

Results

Amplification curve
See Appendix A

Cq results reported by real-time thermal cycler

Sensor	Cq	Specifications	Pass/Fail
1	17.50	N/A	N/A
2	17.65	N/A	N/A
3	17.15	N/A	N/A
4	17.68	N/A	N/A
5	17.36	N/A	N/A
6	17.19	N/A	N/A
7	17.43	N/A	N/A
8	17.68	N/A	N/A
9	17.40	N/A	N/A
10	17.15	N/A	N/A
11	17.58	N/A	N/A
12	17.44	N/A	N/A
13	17.37	N/A	N/A
14	17.39	N/A	N/A
15	17.21	N/A	N/A
Average	17.41		
Standard deviation	0.18		
Uniformity (max - min)	0.53		

Color filter: FAM

Recommended control positions

Positive	A4	H1
Negative	D7	A10

Appendix A

DUT: Device under test
The device under calibration which is the real-time thermal cycler.

CU: Calibration Unit
The measurement system which is used for execution of the calibration (thermal and/or optical).

Cq: Amplification curve
Amplification curve = Quantification cycle. Cycle in which real-time PCR growth curve crossed the threshold.

During the amplification curve simulation, the real-time thermal cycler detects the light which is emitted by the optical calibration system and calculates Cq values for each channel. The light intensity is identical for each channel and gradually increased from 20 % to 100 %. The emitted light is independent of the temperature reached by each individual well.
The Cq value of each channel is determined by the DUT. In an idealized DUT the reported Cq values of all channels are identical and the uniformity is equal to 0 (zero).
A delta Cq of 1 represents a factor 2 difference in the DNA amount.

Generic reference value for each channel: $-1 \leq Cq_{avg} \leq 1$
(value can be used for normalization)

Terminology

Uncertainty [°C]	Parameter associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the quantity intended to be measured.
Cq	Quantification cycle. Cycle in which real-time PCR growth curve crossed the threshold.
Uniformity [°C]	Maximum temperature minus minimum temperature at a specific time.
Uniformity Cq	Maximum Cq minus minimum Cq as determined by the DUT.

Calibration information

Calibration number	**OA75012402-BY73**
Software version	**Cyclertest Measurement Software 1.0.5.23629**
Engineer	**Stephanie Bracht (Kalibrierlabor)**
Data filename	**A75012402-BY73_20260615142447.cl2**
Sample interval	**250ms**
QMS document	**TBD**
Measurement system uncertainty	**0.25 °C (k=2)**

Equipment information (CU)

Probe serial	**A75012402**
Probe Type	**GSOC Optical 96v15Plus4**
Probe calibration date	**31-Jul-2025**
Probe calibration expire date	**31-Jul-2026**
Hardware box serial	**A75012402**
Hardware box version	**0x0625**
T-RH device serial	**N/A**
T-RH device calibration date	**N/A**

Disclaimer

License of product usage

Control code

Go to https://core.cyclertest.com/code/ to verify the data on this certificate.
Code: KS5CZ-BZSX6-3E2RR-HDN8M-UYYHJ

Or scan the QR code to go to the page direcly.