WHITE PAPER

Thermocycler for Automated PCR

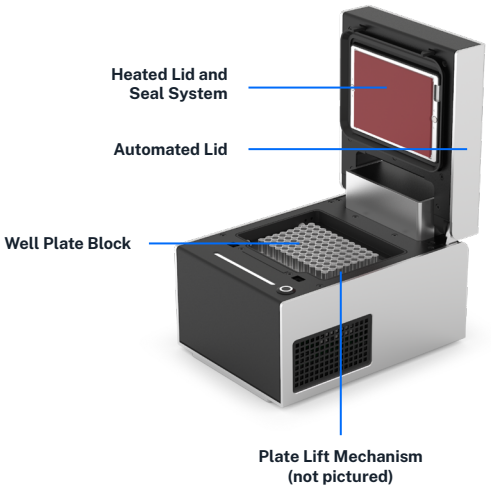
The Opentrons Thermocycler GEN2 delivers accurate and uniform amplification with an exceptionally low evaporation rate.

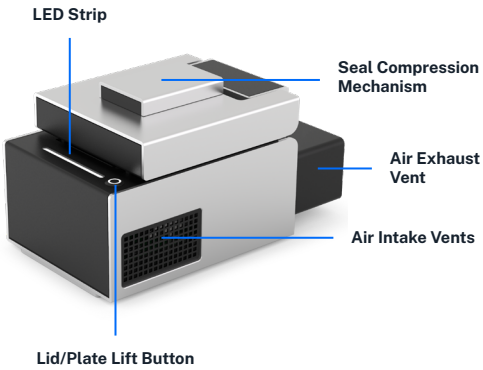
Written by
Opentrons

Product Description

The Opentrons Thermocycler Module is a fully automated on-deck thermocycler, providing hands-free PCR in a 96-well plate format. It is compatible with all Opentrons robots and software to allow for fully automated PCR reaction setup and thermocycling on your Opentrons robot.

The automated, heated lid and disposable seal fit tightly over the plate, ensuring efficient sample heating and minimal evaporation. On completion of thermocycling, the plate lift mechanism enables easy removal of PCR plates via hand or robotic gripper. The air exhaust vent removes hot air from the thermocycler and directs it away from the robot deck.





THERMOCYCLER SPECIFICATIONS	
Compatible Plates	Full-skirted, rigid, 96-well, 0.2 mL PCR plates (recommended: Opentrons Tough PCR Plates)
Working Volume	10–100 µL
Thermal Block Temperature Range	4–99 °C
Thermal Block Maximum Heating Ramp Rate	4.25 °C/s between ambient and 95 °C
Thermal Block Maximum Cooling Ramp Rate	2.0 °C/s between 95 °C and ambient
Heated Lid Temperature Range	37–110 °C
Programmability	Python API & Protocol Designer

Performance Data

Opentrons conducted a series of rigorous performance tests on a statistically significant sample of Thermocycler GEN2 units to demonstrate quality and adherence to industry-standard performance specifications.

TABLE 1

Accurate and Uniform Temperature at Steady-State

TARGET (°C)	EXPECTED UNIFORMITY (°C)	UNIFORMITY [20 µL] (°C)	UNIFORMITY [100 µL] (°C)	EXPECTED ACCURACY (°C)	AVG TEMP [20 µL] (°C)	AVG TEMP [100 µL] (°C)
4	± 1	± 0.19	± 0.306	± 1	4.071	3.887
20	± 0.4	± 0.233	± 0.334	± 0.3	20.219	20.249
55	± 0.4	± 0.19	± 0.209	± 0.3	55.088	55.096
72	± 0.4	± 0.237	± 0.218	± 0.3	72.114	72.095
95	± 0.5	± 0.267	± 0.257	± 0.3	95.175	95.147

Table 1. Steady-state temperature uniformity and accuracy results.
The uniformity and accuracy across eight different thermocycler units were assessed at five target temperatures for volumes of 20 µL and 100 µL. Both uniformity and accuracy measurements were within expected range at each target temperature.

QUICK RAMP RATE

The average maximum ramp rate during heating (to 99 °C) and cooling (to 4 °C) across eight different units was determined. Each setpoint had a hold time of 5 minutes. The thermocycler demonstrated a maximum average ramp rate of 4.45 °C/s **(Figure 1)**.

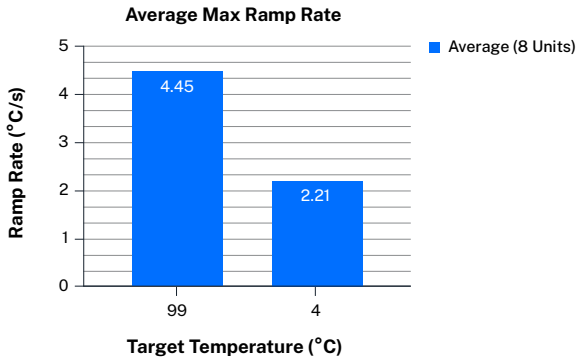


Figure 1. Ramp rate test results.

Performance Data

The uniformity of amplification across PCR plates was tested with reusable seals, using plates filled with variable reaction volumes, and using plates that were filled on the OT-2 robot.

Lambda DNA was amplified using Lambda primers with the following thermocycling parameters: an activation step at 94 °C for 10 minutes, 25 cycles of amplification at 94 °C for 15 seconds and 70 °C for 1.5 minutes, and a final extension at 72 °C for 7 minutes. Amplification was measured for each well using raw fluorescence values captured by a plate reader.

The coefficient of variation (CV%) was calculated **(Table 2)**. Data indicates that seals can be reused up to 25 times without a reduction in performance. The CV% remained low for both 10 µL and 100 µL reaction volumes. Plate filling experiments using the OT-2 robot yielded low CV% showing that a wide range of working volumes can be reproducibly amplified.

TABLE 2

Uniform Amplification

APPLICATION	AVERAGE CV (%)	SEAL REUSE NUMBER	REACTION VOLUME (µL)
Reusability	2.91	1	10
Reusability	2.76	10	10
Reusability	3.04	25	10
OT-2 Plate Fill	3.82	1	10
OT-2 Plate Fill	3.37	1	100

Table 2. Amplification uniformity results.

LOW EVAPORATION RATE

The evaporation rate was assessed across 50 different thermocycler units for sample volumes of 10 µL, 20 µL, 50 µL and 100 µL in 96-well PCR plates. Sample volume weight was measured before and after a 3-step PCR run consisting of 35 cycles with the following setpoints and respective hold times: 94 °C (10 s), 70 °C (30 s), and 72 °C (30 s).

The average evaporation rate was <6% for all tested sample volumes and <1% for the maximum sample volume (100 µL) **(Figure 2)**.

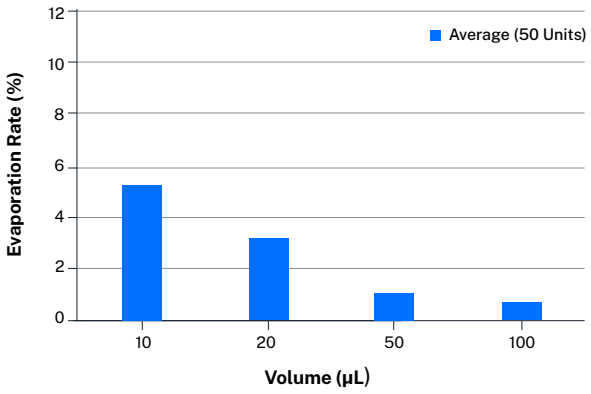


Figure 2. Evaporation test results.

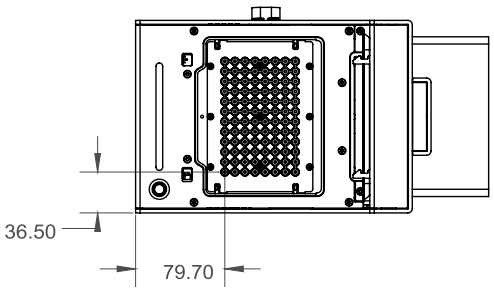
Data indicates that the Opentrons Thermocycler Module GEN2 provides consistent and reliable amplification with minimal sample volume losses for reaction volumes as low as 10 µL. This model demonstrates quick temperature ramping and the ability to hold temperatures at a steady state for a wide range of setpoints. Amplification across a 96-well plate proved to be uniform even with reuse of disposable seals, variable reaction volumes, and preparation of the sample plate both on- or off-robot. The Opentrons Thermocycler Module GEN2 provides repeatable and accurate amplification results across a 96-well PCR experiment and between instruments for day-to-day consistency in the laboratory.

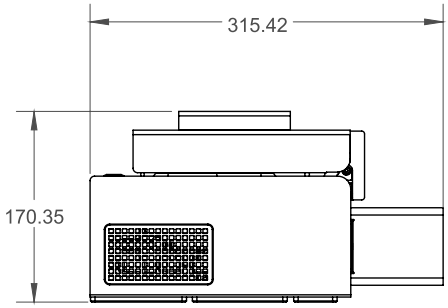
Dimensional Drawings

Millimeter units are shown in all dimensional drawings.

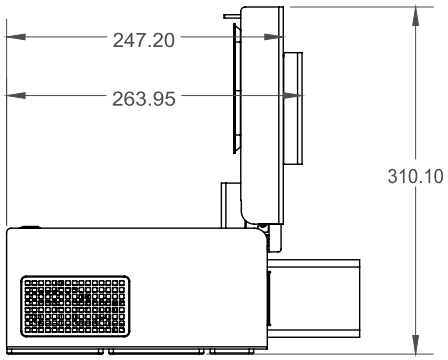
THERMOCYCLER GEN2 MODULE

Mass: 8.4 kg





LID CLOSED



LID OPEN