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CFX Opus 96 Real-Time PCR Detection System

Catalog #12011319 and #12013758 (CFX Maestro V 2.0 Software)

Specifications for Reference Use in Sole-Source Justifications

Bio-Rad Laboratories, Inc. is the original manufacturer and sole supplier of the CFX Opus 96 real-time PCR detection system.

The CFX Opus 96 system can generate a thermal gradient across the 96-well reaction block to support rapid optimization of real-time PCR assays. The CFX Opus 96 system is compatible with many fluorescent technologies for real-time PCR amplification. The System can capture up to five fluorophores in a single reaction well using "All Channels" scan mode, or, for faster scanning time, one fluorophore when using the SYBR/FAM or FRET Scan Modes. CFX Maestro™ software supports data acquisition, viewing, and analysis of data generated by the CFX Opus 96 system and supports real-time PCR applications such as PCR quantification, melt curve analysis, end point analysis, allelic discrimination, and gene expression analysis.

Design and performance specifications of the CFX Opus 96 real-time PCR detection system:

1. System can run real-time PCR experiments without being attached to a computer. When operated as a stand-alone instrument, the instrument will save at least 1000 run files.
2. Real-time data traces can be viewed during a run from the thermal cycler base or on CFX Maestro (if a computer is connected to the instrument).
3. System includes an automated lid that opens, closes, and can apply sealing force to reaction wells.
4. System does not have lid or drawer that extends beyond the footprint of the system nor requires additional operating clearance. *
5. System can detect a 1.3-fold change in gene expression. *
6. Two new pending patents for heat pump and thermal management (BRP01044-US-PROV-01 and BRP01106-US-PROV-01). *
7. Thermal gradient enables optimization of multiple temperatures in a single assay. Temperature differences of up to 24°C front-to-back can be created. *
8. Peltier-driven thermal cycler features a maximum ramping speed of 5°C/sec, with an average ramp rate of 3.3°C/sec.
9. Has a thermal block operational range of 4–100°C.
10. Sample block temperature accuracy is +/-0.2°C of programmed target at 90°C, with a uniformity of +/-0.3°C well-to-well within 10 seconds of arrival at 90°C.
11. Optical system allows excitation and detection of up to five fluorescent dyes in a single reaction well.
12. Optics shuttle independently illuminates and detects fluorescence from each well with the same LED/detector pair per channel. The system uses six filtered LEDs for illumination and differentially detects emission using six filtered photodiodes (one for each channel plus FRET). *
13. Fixed optical path, directly over each well, eliminates the need to normalize for positional bias.
14. Optics shuttle excites fluorescent dyes with absorption spectra in the 450–684 nm range; optimally detects dyes with emission spectra in the 515–730 nm range.
15. One channel is dedicated for FRET and Protein Thermal Shift (Protein Melt) experiments — illuminate dyes with absorption spectra in the 450–490 nm range and detect dyes with emission spectra in the 560–580 nm range.
16. System reads all 96 wells with all channels within 12 seconds.
17. In "SYBR/FAM" scan mode, the system reads all 96 wells within 3 seconds.
18. Has a linear dynamic range of 10 orders of magnitude for detection of input samples.

19. Detects one copy of target sequence in human genomic DNA.
20. Reaction volumes from 1–50 μ L can be used, 10–50 μ L recommended.
21. Detects ≤ 10 fmol of fluorescein.
22. System can be integrated with an automation system for hands-free operation. API is available for use in integrations.
23. Will continue to run and complete a run if the software is stopped or interrupted. This prevents run data from being lost in case of unintentional interruption of the software. This function is especially valuable for precious or limited samples, whereby all data is not lost for a run that was interrupted. *
24. System can directly connect to a network file storage location through Wi-Fi or ethernet with user credentials and can read and write, given system access rights.
25. System can connect to BR.io cloud platform for remote monitoring, experiment set up, and Cq analysis.

CFX Maestro Software specifications:

1. Multiplex amplification and melt curve and end-point analyses can be performed on up to five fluorophores in a single reaction well.
2. Multiple plates can be combined into one experiment with the Gene Study Feature.
3. Additional thermal cycler repeat steps can be added to a GOTO loop.
4. Run results can be displayed in a customizable configuration so that multiple panes of information may be viewed in a single window. *
5. Multiple standard curves can be simultaneously viewed when a common fluorophore is used for multiple targets.
6. PCR quantification by standard curve features automated calculations of reaction efficiencies with y-intercept.
7. Automated allelic discrimination can use end-point fluorescence or quantification cycle (Cq) values.
8. Gene expression analysis can be performed by relative quantity (Δ Cq) or normalized expression ($\Delta\Delta$ Cq).
9. If reaction efficiencies are present, these will be automatically utilized in gene expression analysis.
10. Multiple reference genes can be assigned in normalized expression ($\Delta\Delta$ Cq) analysis.
11. Individual reaction efficiencies can be used in normalized expression ($\Delta\Delta$ Cq) analysis.
12. Reference Gene Selector Tool displays gene stability for selection of ideal reference genes. *
13. Up to 5,000 Cq values from different data files can be compared for gene expression analysis.
14. Multiplex data can be displayed for up to five fluorophores.
15. Data graphs and tables can be exported into Microsoft Excel or printed from software directly.
16. License for qbasePLUS software permits further gene expression analysis. *
17. Embedded reports tool can be customized by the user to export run information, data tables, graphs, and analysis parameters in a specified order to PDF or other file formats. *
18. Exported data type and format can be customized.
19. Integrate with any laboratory information management system (LIMS) using built-in LIMS file management.
20. Optional CFX Maestro Software, Security Edition provides secure user log-in, detailed audit trail reporting, and electronic record-keeping tools to assist compliance with U.S. FDA 21 CFR Part 11 regulations.
26. The software can be set up to email run files to a specified user after runs are completed.
27. Help information is contextual to the active window in the software.
28. Software enables data to be grouped and interpreted by both technical replicates and biological groups*
29. Image export options include DPI selection up to 600DPI, choice of any image size, and color selection using RGB specifications. *

- 30. Image annotation function allows for automatic p-value annotation and addition of arrows, circles, and text directly onto graph images. *
- 31. Software performs t-tests and 1-way ANOVA calculations. *
- 32. Software integrates with PrimePCR for run setup and results analysis. PrimePCR Controls Analysis tool provides interpretation of controls in a run such as positive control, reverse-transcriptase check, and genomic DNA contamination. *
- 33. Software displays data in multiple formats including bar chart, dot plot, box-and-whisker plot, scatter plot, clustergram, and volcano plot.
- 34. Custom data view allows a user to define the specific information to be displayed in a single view, allowing a user to mouse over a well and see that specific result data. *
- 35. Control up to 4 Instruments with one PC running CFX Maestro. *

*Unique to Bio-Rad CFX

Normalized expression Calculations used are comparable to $\Delta\Delta Cq$ but utilize more sophisticated calculations to have multiple normalizers. See <https://youtu.be/TWmu7IXrvIE> for specific walk through analysis calculations.