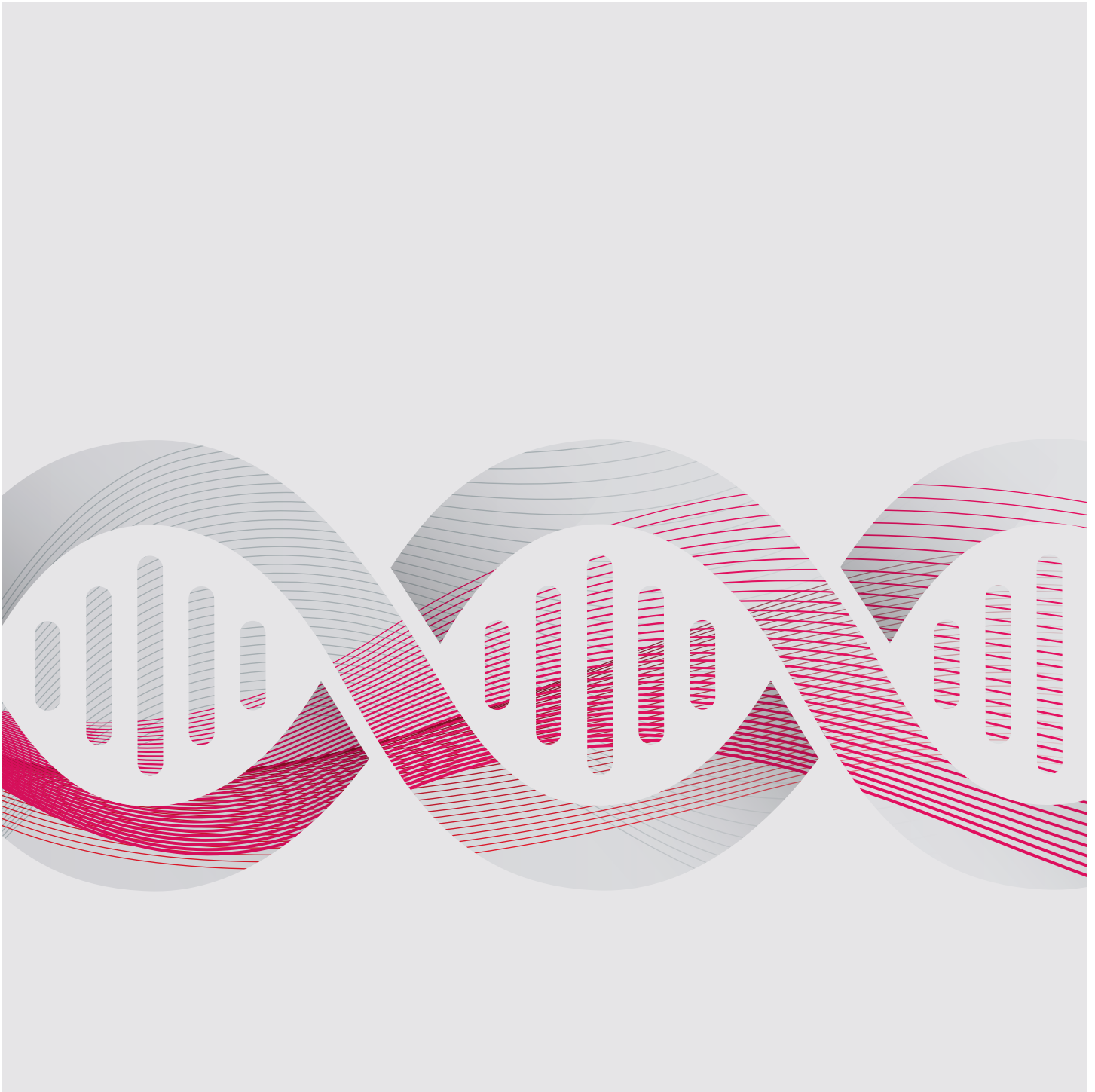# CFX Duet Real-Time PCR System

Instrument Guide

BIO·RAD

CFX Duet Real-Time PCR Detection System

Instrument Guide

For Research Use Only

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Bio-Rad Technical Support

The Bio-Rad Technical Support department in the U.S. is open Monday through Friday, 5:00 AM to 5:00 PM, Pacific Time.

Phone: 1-800-424-6723, option 2

Email: Support@bio-rad.com (U.S./Canada Only)

For technical assistance outside the U.S. and Canada, contact your local technical support office or click the Contact us link at www.bio-rad.com.

Notice

Revision History

Document	Date	Description of Change
CFX Duet Real-Time PCR Detection System Instrument Guide (Doc ID #10000148048)	December 2021	Initial Release

Table of Contents

Table of Contents

Safety and Regulatory Compliance

The CFX Duet Real-Time PCR Detection System (known in this guide as CFX Duet) heats and cools very quickly during operation. Bio-Rad strongly recommends that you follow the safety specifications listed throughout this guide.

Note: Use only Bio-Rad–supplied USB cables (catalog # 16007721) when using the CFX Duet instrument.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Table 1 defines each safety warning label.

Table 1. Meaning of safety warning labels

Icon	Meaning
⚠️	Operating the CFX Duet instrument before reading this manual can constitute a personal injury hazard. For safe use, do not operate this instrument in any manner unspecified in this manual. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument. Always handle all components of the system with care and with clean, dry hands.
☣️	**Warning about handling biohazardous materials** When handling biohazardous samples, adhere to the recommended precautions and guidelines and comply with any local guidelines specific to your laboratory and location.
🔥	**Warning about risk of burning** A thermal cycler generates enough heat to cause serious burns. Wear safety goggles or other eye protection at all times during operation. Always allow the sample block to return to idle temperature before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns.
💥	**Warning about risk of explosion** The sample blocks can become hot enough during the course of normal operation to cause liquids to boil and explode.

Safety and Regulatory Compliance

Safety Compliance

The CFX Duet instrument has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General requirements

- IEC 61010-2-010:2019 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-010: Particular requirements for laboratory equipment for the heating of materials

- IEC 61010-2-081:2019 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

- CAN/CSA-C22.2 NO. 61010-1-12:2018 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General Requirements

- CAN/CSA-C22.2 NO. 61010-2-010:19 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 2-010: Particular requirements for laboratory equipment for the heating of materials

- CAN/CSA-C22.2 NO. 61010-2-081:19 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

- EN 61010-1:2010 Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General requirements

- EN 61010-2-010:2014 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-010: Particular requirements for laboratory equipment for the heating of materials

- EN 61010-2-081:2015 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

- UL 61010-1:2012 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 1: General Requirements

- UL 61010-2-010:2019 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-010: Particular requirements for laboratory equipment for the heating of materials

- UL 61010-2-081:2019 Safety requirements for electrical equipment for measurement, control and laboratory use — Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes

Electromagnetic Compatibility (EMC)

The CFX Duet instrument has been tested and found to be in compliance with all applicable requirements of the following electromagnetic compatibility standards:

- IEC 61326-1:2012 Electrical equipment for measurement, control and laboratory use — EMC requirements — Part 1: General requirements. Tested as a Class A device

- EN 61326-1:2013 Electrical equipment for measurement, control and laboratory use — EMC requirements — Part 1: General requirements. Tested as a Class A device

- FCC Part 15, Subpart B, Sections 15.107 and 15.109. Tested as a Class A digital device

- CAN ICES-003v6:2019 Interference-causing equipment standard, information technology equipment (including digital apparatus) — Limits and methods of measurement. Tested to Class A limits

EMC Warnings and Notes

- **Warning:** Changes or modifications to this unit, not expressly approved by Bio-Rad, could void the user's authority to operate the equipment.

- **Note:** This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at their own expense.

- **Note regarding FCC compliance:** Although this instrument has been tested and found to comply with Part 15, Subpart B of the FCC Rules for a Class A digital device, please note that this

compliance is voluntary, for the instrument qualifies as an "exempted device" under 47 CFR 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture.

■ **Note regarding cables:** This instrument was tested for EMC compliance using specially designed USB cables, which are supplied with the instrument. These cables, or Bio-Rad authorized replacements, must be used with this instrument to ensure continued compliance with the EMC emissions limits.

Environment Requirements

The CFX Duet has been designed to be safely operated under the environmental conditions listed in the following table.

Table 2. environment requirements

Parameter	Specification
Environment	Indoor use only
Operating altitude	Up to 2,000 meters above sea level
Ambient room temperature	15–31°C*
Transport and storage temperature	–20° to 60°C** –4 to 140°F
Relative humidity	20% to 80% (noncondensing)***
Operating power	100 to 240 VAC ±10%, 50/60 Hz, 850 W Max
Mains supply voltage fluctuation	±10%
Maximum power usage	<850 watts
Fuses	10 A, 250 V, 5 x 20 mm, fast blow (qty. 2)
Overvoltage category	II
Pollution degree	2

*Operating the instrument outside of this temperature range may not meet performance specifications. A room temperature between 5–40°C is considered safe.

**Store and transport the instrument in its shipping container to meet these temperature conditions.

***Operating the instrument at 4ºC should be limited to 18 hours at these conditions. Holds at 4ºC can be performed for up to 72 hours if humidity is less than 60% (noncondensing).

Hazards

The CFX Duet instrument is designed to operate safely when used in the manner prescribed by the manufacturer. If the system or any of its associated components is used in a manner not specified by the manufacturer, the inherent protection provided by the instrument may be impaired. Bio-Rad is not liable for any injury or damage caused by the use of this equipment in any unspecified manner, or by modifications to the instrument not performed by Bio-Rad or an authorized agent. Service of the CFX Duet instrument should be performed only by trained Bio-Rad personnel.

Biohazards

The CFX Duet instrument is a laboratory product. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

Note: No biohazardous substances are exhausted during normal operations of this instrument.

General Precautions

- Always wear laboratory coat, laboratory gloves, and safety glasses with side shields or goggles.

- Keep your hands away from your mouth, nose, and eyes.

- Completely protect any cut or abrasion before working with potentially infectious materials.

- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.

- Remove wristwatches and jewelry before working at the bench.

- Store all infectious or potentially infectious material in unbreakable leak-proof containers.

- Before leaving the laboratory, remove protective clothing.

- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves.

- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.

- Do not expose materials that cannot be properly decontaminated to potentially infectious material.

- Upon completion of an operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach).

Surface Decontamination

WARNING! To prevent electrical shock, always turn off and unplug the instrument prior to performing decontamination procedures.

The following areas can be cleaned with any hospital-grade bactericide, virucide, or fungicide disinfectant:

■ Outer lid and chassis

■ Inner sample block surface and sample block wells

To prepare and apply the disinfectant, refer to the instructions provided by the product manufacturer. Always rinse the sample block and sample block wells several times with water after applying a disinfectant. Thoroughly dry the sample block and sample block wells after rinsing with water.

Important: Do not use abrasive or corrosive detergents or strong alkaline solutions. These agents can scratch surfaces and damage the sample block, resulting in loss of precise thermal control.

Disposal of Biohazardous Material

Dispose of the following potentially contaminated materials in accordance with laboratory local, regional, and national regulations:

■ Clinical samples

■ Reagents

■ Used reaction vessels or other consumables that may be contaminated

Chemical Hazards

The CFX Duet instrument contains no potentially hazardous chemical materials.

Explosive or Flammability Hazards

The CFX Duet instrument poses no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad Laboratories.

Electrical Hazards

The CFX Duet instrument poses no uncommon electrical hazard to operators if installed and operated properly without physical modification and connected to a power source of proper specification.

Transport

Before moving or shipping the CFX Duet instrument, decontamination procedures must be performed. Always move or ship the system in a separate container in the Bio-Rad-supplied packaging material, which protects the system from damage.

For information about transporting the system and to request the appropriate packaging material, contact your local Bio-Rad office.

Battery

The CFX Duet instrument uses one 3 V lithium-metal coin cell battery to maintain time settings in the event of AC power loss. If the time does not remain set after the unit is turned off, it may be an indication that the batteries are getting weak.

WARNING! Do not attempt to change the batteries. They are not user serviceable. Instead, contact Bio-Rad Technical Support for assistance.

For the State of California, USA only

- Perchlorate material — Lithium batteries contain perchlorate material; special handling may apply. See www.dtsc.ca.gov/hazardouswaste/perchlorate.

Warranty

The CFX Duet instrument and its associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad office for the details of the warranty.

Chapter 1 Introduction

The CFX Duet Real-Time PCR Detection System (known in this guide as CFX Duet) is a real-time PCR system with an integrated 96-well sample block.

When used with the CFX Maestro Software (known in this guide as CFX Maestro), CFX Duet becomes a powerful real-time PCR detection system for researchers who run polymerase chain reaction (PCR) for sequencing, cloning, gene expression studies, mutagenesis, and many other applications.

This guide explains how to set up CFX Duet to perform sensitive quantitative PCR.

Important: The CFX Duet instrument requires the latest version of CFX Maestro to perform PCR runs. You will not be able to perform PCR runs on the CFX Duet instrument without CFX Maestro.

For instructions on how to use CFX Duet to perform PCR experiments and analyze PCR data, refer to the CFX Maestro User Guide.

Main Features

The main features of CFX Duet include:

- USB ports enable direct connectivity to a computer running CFX Maestro

- Optical shuttle technology that does not require ROX or another normalizer

- Programmable temperature gradient to quickly and easily identify optimal annealing temperatures

- Uniform thermal performance across the reaction block

- Two channel optics for simultaneous dual target detection

- FRET mode for protein characterization workflows.

Performance Specifications

CFX Duet components provide sensitive detection for precise quantification and target discrimination. The tables in this section list the sample block and optical detection performance specifications for the CFX Duet instrument.

Sample Block Performance Specifications

Table 3. CFX Duet Real-Time PCR Detection System sample block specifications

Item	Specification
Volume	1–50 µl (10–50 µl Recommended)
Maximum ramp rate	5°C/sec
Average ramp rate	3.3°C/sec
Lid temperature	30–110°C
Heating and cooling method	Peltier
Temperature range	4–100°C
Increment	-10°C to 10°C/cycle
Gradient	
Operational range	30–100°C
Programmable span	1–24°C
Temperature accuracy	±0.2°C of programmed target at 90°C
Temperature uniformity	±0.3°C well-to-well within 10 sec of arrival at 90°C

Optical Detection Performance Specifications

Table 4. CFX Duet Real-Time PCR Detection System optical detection specifications

Item	Specification
Excitation	3 filtered LEDs
Detection	3 filtered photodiodes
Range of excitation/emission wavelengths	450–580 nm
Multiplex analysis	2 targets per well
Scan time	
All channels	12 sec
Single-channel fast scan	3 sec
FRET	Yes
Sensitivity	Detects 1 copy of target sequence in human genomic DNA
Dynamic range	10 orders of magnitude

Finding Out More

You can access the CFX Maestro User Guide, which describes the software's full features, in the Help Menu of the CFX Maestro Home Window. You can also access the Maestro User Guide by logging onto bio-rad.com and searching for catalog #10000126764.

Note: Click the Bio-Rad logo in the upper right corner of any CFX Maestro window to launch Bio-Rad's website. This site includes links to technical notes, manuals, product information, and technical support. This site also provides many technical resources on a wide variety of methods and applications related to PCR, real-time PCR, and gene expression.

The CFX Duet Real-Time PCR Detection System web site (bio-rad.com/CFXDuet) provides access to technical notes, manuals, product information, and technical support. This site also provides many technical resources on a wide variety of methods and applications related to real-time PCR.

Chapter 3 Setting Up the CFX Duet Real-Time PCR Detection System

This chapter explains how to set up the CFX Duet instrument at your site.

Tip: Before setting up the CFX Duet instrument, familiarize yourself with the system, its ports, and accessories.

Site Requirements

The tables in this section list the bench space and power requirements necessary to successfully install and use the CFX Duet instrument. For weight and dimensions of the packaged instrument, see Packaged Instrument Specifications on page 26.

Note: Install your CFX Duet instrument on a flat, dry surface with sufficient cool airflow for it to run properly.

Bench Space Requirements

Caution: The CFX Duet instrument requires at least 10 cm behind and at least 5 cm along one side or the top to allow exhaust air to flow properly.

Additionally, ensure you provide sufficient space around the system to disconnect the power cord or to access the power switch in case of an emergency.

Note: While setting up the CFX Duet instrument, ensure that you have sufficient space near the system for the computer running CFX Maestro.

Table 5. CFX Duet Real-Time PCR Detection System bench space requirements

Item	Specification
Dimensions	W: 13 in; 33 cm
	D: 22 in; 56 cm
	H: 14 in; 36 cm
Weight	48 lb; 22 kg

Power Requirements

Power to the CFX Duet instrument must be stable and within specifications to ensure proper operation. The power cord connected to the power inlet port must be rated for 10 amps or more.

Table 6. CFX Duet Real-Time PCR Detection System power requirements

Item	Specification
Fuses	10 A, 250 V, 5 x 20 mm, fast blow (qty. 2)
Number of power sockets	■ One socket for the CFX Duet instrument
	■ One socket for the computer running CFX Maestro

System Overview

The illustrations in this section display the main components of the CFX Duet instrument.

Front View

LEGEND

1. Sample block	2. Retractable lid (partially retracted)
3. Air intake vents	4. LED system status light

Details

■ **Sample block** — holds the 96-well plate.

■ **Retractable lid** — provides access to the sample block.

   Note: The CFX Duet instrument does not have a manual open/close button on the lid. To open or close the lid, use the lid control in CFX Maestro.

■ **LED system status light** — indicates system status:

   □ Green (constant) — run is in progress.

   □ Green (flashing) — run is paused.

   □ Blue (flashing) — run has completed (flashes until lid has opened).

   □ Blue (flashes for ten seconds) — system is in use (indicates that the instrument is selected in CFX Maestro).

□ Red (flashing) — critical system error.

□ White — system is idle (not executing a run).

Important: The system will become idle (indicated by a white LED) if the CFX Duet instrument disconnects from CFX Maestro.

□ OFF — system is shut down.

■ **Air intake vents** — allow the system to heat and cool quickly.

Important: Keep all air vents clean and clear of obstruction. Do not insert any object in the air vents at any time. Fans or other internal moving parts may collide with the object and cause personal injury or damage the instrument. Conductive objects could contact internal circuitry and cause damage to the instrument.

Back View
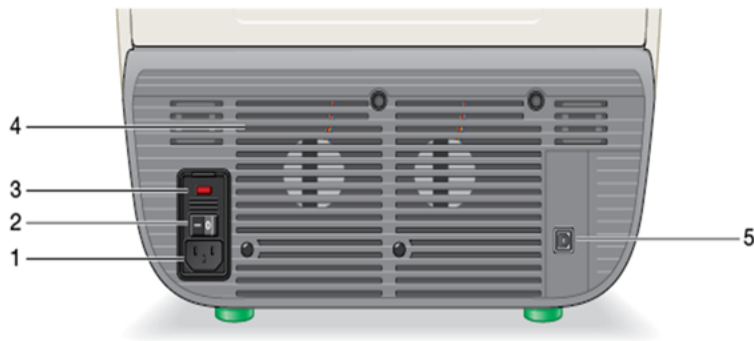
LEGEND

1. Power input	2. Power switch
3. Fuses	4. Cooling vents

5. USB Type B port

Details

- **Power input** — connects to AC power.

- **Power switch** — turns the CFX Duet instrument power on or off.

- **Fuses** — provides access to the fuses.

- **Cooling vents** — cool the instrument.

  Important: Do not block the cooling vents. For optimal operation, ensure that air can circulate behind the instrument.

- **USB Type B port** — connects the CFX Duet instrument to a computer running CFX Maestro.

Unpacking and Starting the Instrument

This section explains how to properly unpack and start the CFX Duet instrument. Read this section before you begin.

Caution: The packaged CFX Duet instrument weighs approximately 57 lb (26 kg). Bio-Rad strongly recommends that you use a pallet jack to move the instrument from the shipping dock to the laboratory.

Important: Use proper lifting techniques when moving and lifting the instrument to prevent damage to the instrument and personal injury. Bio-Rad recommends that two or more people lift the instrument.

Packaged Instrument Specifications

Table 7 lists the specifications of the packaged CFX Duet instrument. For information about bench space specifications, see Site Requirements on page 21.

Table 7. Specifications for the packaged CFX Duet Real-Time PCR Detection System

Parameter	Specification
Weight	57 lb; 26 kg
Dimensions	Depth: 28 in; 71 cm
	Width: 19 in; 48 cm
	Height: 19 in; 48 cm

The image below depicts the CFX Duet instrument inside its packaging.
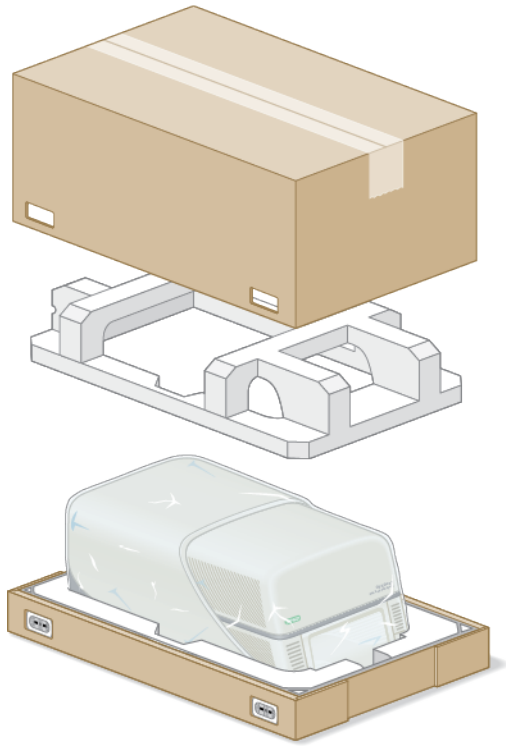
Unpacking the Instrument

Install the CFX Duet instrument on a flat, dry surface with sufficient cool airflow to run properly. The AC power cord is approximately 5 ft. in length. Ensure you have sufficient access to the power outlet before you begin.

Note: Ensure that there is enough clear space on the lab bench for your instrument plus additional space to maneuver it while installing cables and accessories. Additionally, ensure that you have adequate space on the lab bench for the computer running CFX Maestro.

Caution: When handling the instrument, do not grip the retractable lid. To lift the instrument, position your hands underneath the right and left sides of the instrument.

To unpack the system

1. Ensure that the path from the shipping dock to the laboratory is clear of obstacles and can accommodate the packaged CFX Duet instrument and a pallet jack.

2. Using a pallet jack, move the instrument container from the shipping dock to the laboratory.

   Important: Bio-Rad strongly recommends that you do NOT use a hand truck to move the packaged instrument.

3. Release the instrument container next to the lab bench.

4. Using two people, lift and place container onto the lab bench.

5. With scissors or a box cutter, cut and remove the exterior straps that secure the container top to the base.

   Tip: The exterior straps are very tight. Hold the straps securely while cutting to prevent injury.

6. Remove the plastic clips on the bottom corners by firmly pinching the tabs together and pulling the clips straight out.
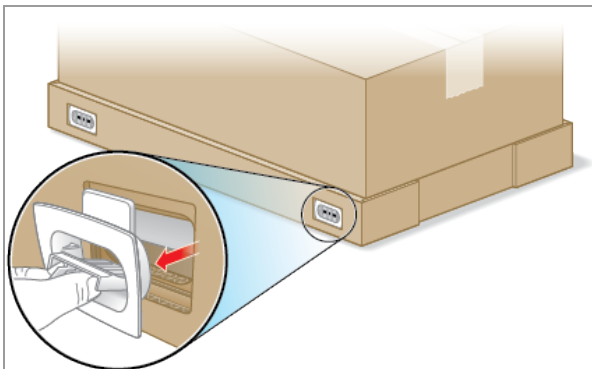
7. Lift the large container top up and out of the base and place it to the side.
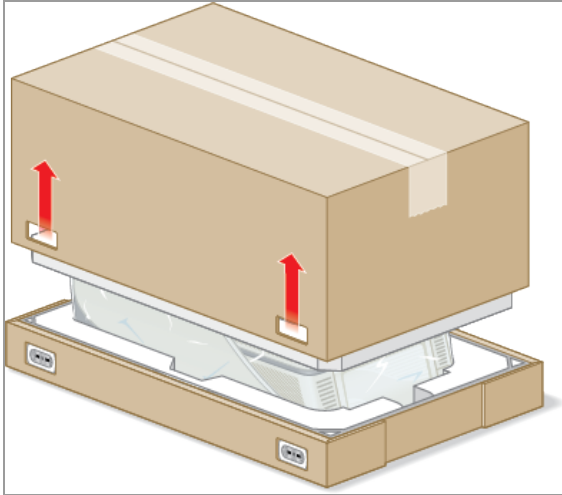
8. Remove the top foam insert and place it to the side.
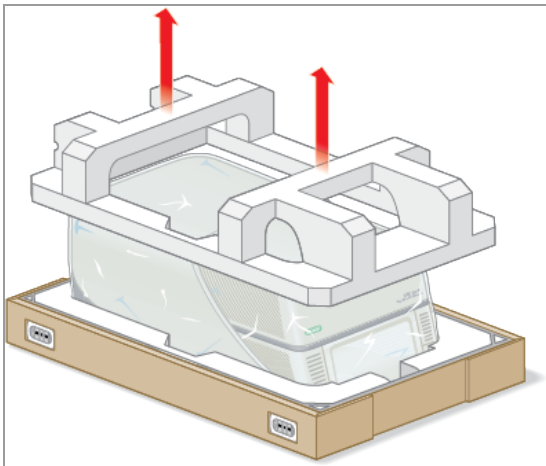
9. Standing in front of the instrument, fold down the front of the cardboard base.
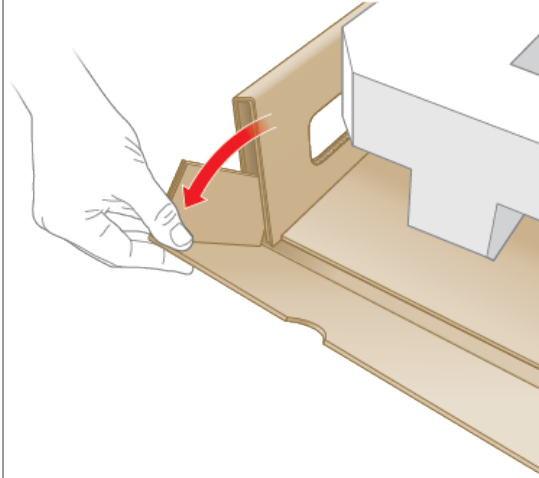
10. While another person holds the base, grasp the cardboard tray and slide the instrument completely out of the base.
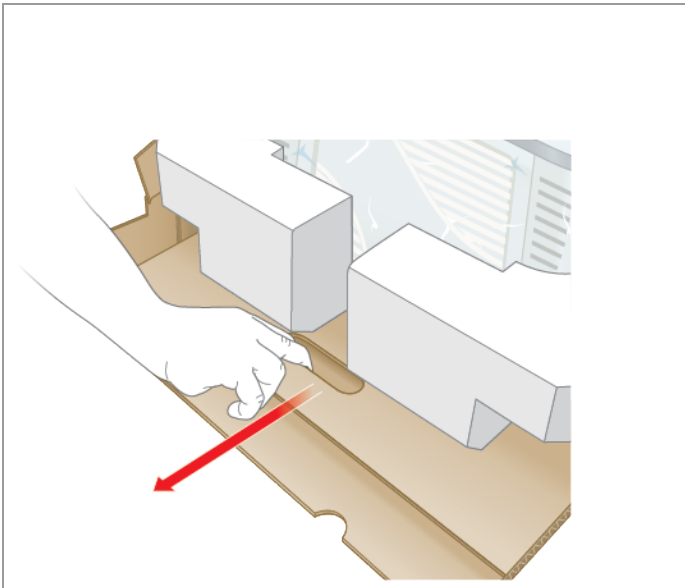
11. Using the lift point on one side of the instrument, gently lift the instrument, remove the foam inserts, and place them to the side.

12. Using two people, carefully lift the instrument and remove the cardboard tray.

13. Slide the plastic cover toward the back of the instrument to remove.

14. Inspect the instrument for any damage.

> **Important:** If you observe any damage to the system, do not continue. Instead, contact Bio-Rad Customer Support.

Connecting the Power Cables and Communication Cables

After you unpack the CFX Duet instrument and install it on the lab bench, you will need to connect the power cord to a power source and a USB Type B cable to a computer running CFX Maestro. This section explains how to connect the power cord and connect to CFX Maestro.

Tip: Before connecting the cables, familiarize yourself with the CFX Duet instrument, its accessory kit, and power switches.

Important: Ensure you have enough room on the lab bench to reach the power switch on the back of the system after connecting the power cord and USB Type B cable. Use only the Bio-Rad-supplied power cord and USB Type B cable.

To connect the CFX Duet instrument power and communication cables

1. Locate the accessory kit that shipped with the CFX Duet instrument.

2. Remove the AC power cord and USB cable from the accessory kit.

   Tip: Store the packing material for future use. If any item is missing or damaged, contact your local Bio-Rad office.

3. Insert one end of the AC power cord into the power inlet port on back of the system.

4. Insert the other end of the AC power cord into an available grounded, surge-protected AC outlet.

5. To connect the CFX Duet instrument to a computer running CFX Maestro, insert the male end of the USB Type B cable into the USB Type B port located on the back of the system. Connect the other end of the USB Type B cable into a USB port on the computer running CFX Maestro.

Important: You must install CFX Maestro before you connect the computer running CFX Maestro to the CFX Duet instrument.

Starting the CFX Duet Real-Time PCR Detection System and Connecting to CFX Maestro

Important: CFX Maestro must be installed on a computer connected to the CFX Duet instrument in order for the software to recognize the instrument. For instructions on how to install and log into CFX Maestro, refer to the CFX Maestro user guide.

To start the CFX Duet Real-Time PCR Detection System

1. Turn on the computer running CFX Maestro.

2. Press the power switch on the back of the instrument to start the CFX Duet instrument.

3. The system performs a series of initialization tests. The CFX Duet instrument then appears in the CFX Maestro Detected Instruments pane.

   Note: If the CFX Duet instrument does not appear in the Detected Instrument pane of CFX Maestro, verify that the USB cable is properly installed. To reinstall drivers, on the Home window in CFX Maestro select Tools > Reinstall Instrument Drivers.

Removing the Shipping Screw

The CFX Duet instrument ships with a red shipping screw inserted in the inner lid to stabilize the optical shuttle during shipping. You must use CFX Maestro to prepare the instrument for screw removal before unscrewing the shipping screw from the CFX Duet instrument.

To remove the shipping screw

1. Locate the beige shipping screw plug that ships with the CFX Duet instrument.

   Tip: This might be located in a plastic sheath taped to the front or side of the instrument.

2. Ensure the power cord is properly inserted into the power input on the back of the instrument.

3. If you have not yet done so, insert the other end of the power cord into an available grounded, surge-protected AC outlet.

4. Press the power switch on the back of the instrument to start the CFX Duet instrument.

5. In CFX Maestro, confirm that the CFX Duet instrument icon appears in the Detected Instruments pane.

   Note: If the CFX Duet does not appear in the Detected Instruments pane of CFX Maestro, see Connecting the Power Cables and Communication Cables on page 31.

6. In the Detected Instruments pane of CFX Maestro, right-click the CFX Duet and select Properties.

7. Select the Shipping Screw tab and click Remove Shipping Screw.

8. Remove the shipping screw from the side of the CFX Duet instrument lid by unscrewing it counter-clockwise.

   Important: You must reinsert the shipping screw should you need to return the instrument for any reason. Save the screw in a safe and accessible place.

   Caution: Do not insert the shipping screw or any other object in the shipping screw hole while instrument is operating. Internal moving parts might collide with the object, which can cause personal injury or damage the instrument.
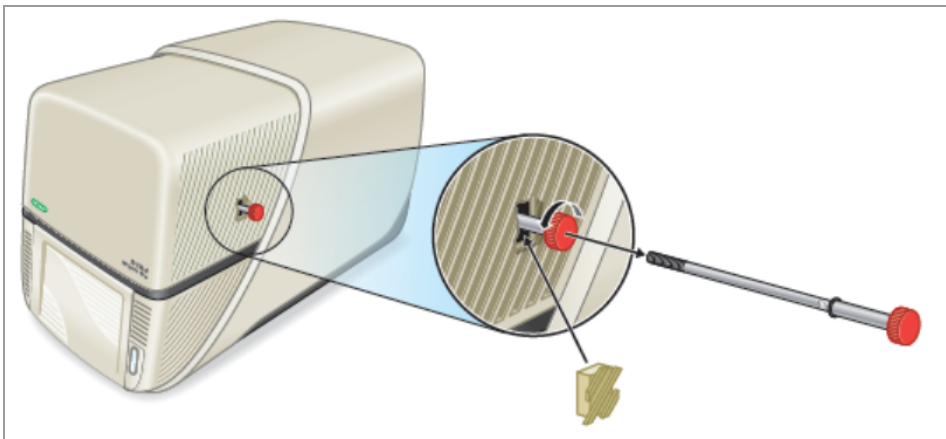
9. In CFX Maestro, click Screw Removed to confirm that the shipping screw is removed.

10. Click Open Lid.

11. Remove the shipping plate from the CFX Duet instrument that protects the sample block.

12. Click Close Lid.

13. Close the Properties dialog box.

Loading the Sample Block

Bio-Rad strongly recommends that you use only low-profile plates and flat-cap tubes with the CFX Duet instrument. Using high-profile plates can crush tubes. Using domed-cap tubes can negatively affect plate reads.

For a list of plates and tubes compatible with the CFX Duet, visit us at www.bio-rad.com/cfxopus or contact your local Bio-Rad Sales representative.

To ensure uniform heating and cooling of samples, reaction vessels must be in complete contact with the sample block. To ensure adequate contact, do the following:

- Confirm that the sample block is clean before loading samples.

- Firmly press the individual tubes, tube strips, or microplates into the block wells.

- When using one or a few tubes, use the tube frame or load at least one empty tube in each corner of the block to ensure the lid exerts even pressure on individual tubes.

Loading Plates, Tubes and Tube Strips into the Sample Block

Caution: Never run a sample with a lid or seal that is open, loose, punctured, or otherwise damaged. Doing so will increase the likelihood of a rupture, which could cause injury or contaminate the instrument.

Important: When running the CFX Duet instrument, always balance the tube strips or add capped tubes to the corner wells to ensure the heated lid applies even pressure across the block.

To load plates into the sample block

1. To open the motorized lid, do one of the following in CFX Maestro:

   - In the Detected Instruments pane, click Open Lid.

   - In the Detected Instruments pane, right-click on the instrument and click Open Lid.

   - On the Start Run tab of the Run Setup pane, click Open Lid.

2. Place the microplate, individual tubes, or tube strips with sealed lids in the block.

   Important: Ensure that the tubes are completely sealed to prevent leakage.

   Tip: For optimal results, load sample volumes of 10–50 µl.

3. For accurate data analysis, verify that the orientation of samples in the block is exactly the same as the orientation of the well contents in CFX Maestro.

4. To close the motorized lid, do one of the following:

   - In the Detected Instruments pane of CFX Maestro, click Close Lid.

- On the Start Run tab of the Run Setup pane of CFX Maestro, click Close Lid.

- In the Detected Instruments pane of CFX Maestro, right click the instrument and click Close Lid.

Important: Ensure that nothing blocks the lid when it closes. Although there is a safety mechanism to prevent the lid from closing if it senses an obstruction, do not place anything in the way of the lid before closing.

Shutting Down the CFX Duet Real-Time PCR Detection System

Follow these instructions to safely and completely shut down the CFX Duet instrument.

To shut down the CFX Duet instrument

1. Ensure that no protocol is running and the instrument is no longer in use.

2. In the CFX Maestro Detected Instruments pane, select the CFX Duet instrument.

3. Click Open Lid.

4. Remove the sample plate located on the block of the CFX Duet instrument.

5. Click Close Lid.

6. Press the power switch on the back panel of the instrument to power down the system.

Chapter 4 Configuring the CFX Duet Real-Time PCR Detection System

After successfully setting up the CFX Duet instrument and installing CFX Maestro, you can configure CFX Maestro (based on the your site's requirements) to perform the following:

- Connect the CFX Duet instrument to a computer running CFX Maestro

- Set the CFX Duet instrument's time zone and local time

- Rename the CFX Duet instrument

- View the CFX Duet instrument's total number of PCR hours and cycles since it was last connected to CFX Maestro

- Set protocol parameters (such as ramp rate, gradient, and temperature)

- Calibrate dyes

For more detailed information on how to perform these tasks, refer to the CFX Maestro User Guide.

Connecting to a Computer Running CFX Maestro Software

During installation, the CFX Maestro software installer automatically installs the instrument drivers onto the computer. When you start the software, CFX Maestro detects the connected instruments.

Important: You must disconnect the CFX Duet instrument from the CFX Maestro computer before you install the software. You do not need to turn off the instrument during the software installation.

To detect connected instruments

1. If you have not yet done so, insert the square (male) end of the supplied USB Type B cable into the USB Type B port located on the back of the CFX Duet instrument.

2. Insert the other (port) end into a USB port on the CFX Maestro computer.

3. If the instrument is not already running, press the power switch on the back of the instrument to turn it on.

4. Start CFX Maestro.

The software automatically detects the connected instrument and displays its name in the Detected Instruments pane in the Home window.
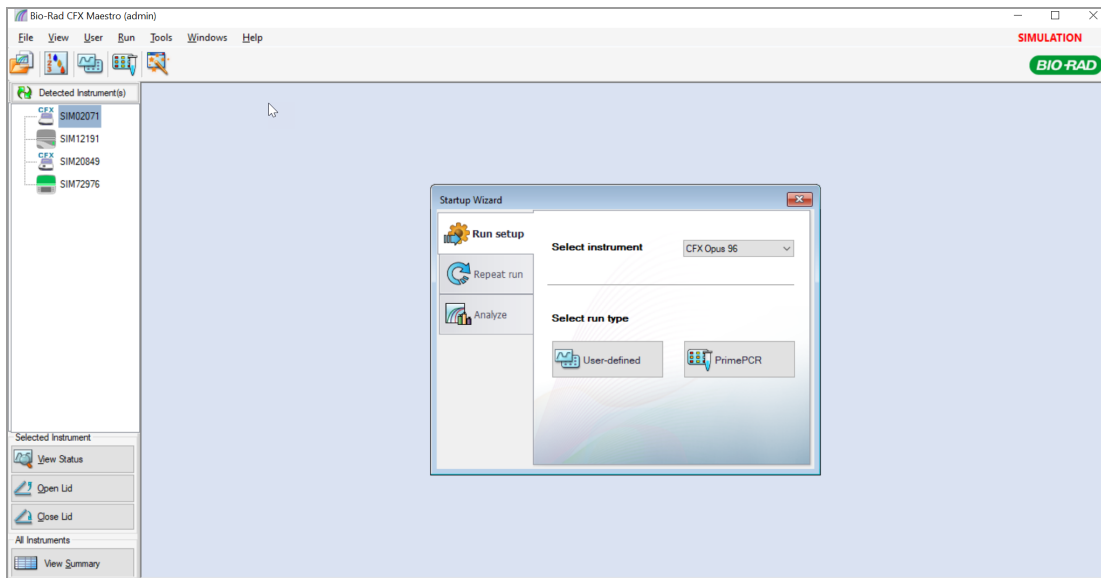
Note: If the CFX Duet instrument does not appear in the Detected Instruments pane, verify that the USB cable is properly installed. To reinstall drivers, select Tools > Reinstall Instrument Drivers in the CFX Maestro software Home window.

Renaming the CFX Duet Real-Time PCR Detection System

You can update the name of the CFX Duet instrument in CFX Maestro so that the instrument is more easily identifiable when performing PCR runs, analyses, and in run reports.

To rename the CFX Duet instrument:

1. Start CFX Maestro.

2. In the Detected Instruments pane, right-click the target instrument and select Properties on the menu that appears.

3. The CFX Duet Instrument Properties dialog box appears.

4. In the Properties tab, type a name in the Rename box at the top of the Properties tab and click Rename.

5. The new name appears in the Nickname row in the Properties tab as well as in the Instrument Properties header bar and the Detected Instruments pane.

Viewing CFX Duet Real-Time PCR Detection System Total Run Hours and Total Cycles

CFX Maestro allows for you to view the Total Run Hours and Total Cycles that the CFX Duet instrument has performed since it was last connected to CFX Maestro.

To view the Total Run Hours and Total Cycles

▶ In CFX Maestro, right-click on the CFX Duet instrument in the Detected Instruments pane, and select Properties to display the Properties dialog. The Total Run Hours and Total Cycles are defined as follows:

■ Total Run Hours at Last Connection – the number of hours that the CFX Duet instrument performed PCR cycles.

■ Total Cycles at Last Connection – the total number of cycles the CFX Duet instrument has accumulated.

Total Run Hours and Total Cycles are updated each time the CFX Duet instrument connects to CFX Maestro.

Total Run Hours and Total Cycles in the CFX Duet Instrument Properties tab:

Property	Current
Properties **Shipping Screw** **Calibrated Dyes**	
Rename your instrument. Use alphabetic characters and numbers (8 characters max). Click Rename button to apply.	SIM26627 Rename
Instrument Name	SIM26627
Model	CFX Duet
Instrument Serial Number	SIM26627
Firmware Versions	
Cycler Module	1.0.0.0
Scanner Module	0.0.0.0
Total Run Hours at Last Connection	3
Total Cycles at Last Connection	125

Parameters and Ranges for Protocol Steps

Create, edit, and run CFX Duet protocols in CFX Maestro. To create, edit, or run a protocol, refer to the CFX Maestro User Guide.

Use the information in Table 8 on page 41 to modify the default settings for the steps in your protocol.

Temperature Steps

The target temperature is a value between 4.0 and 100.0°C, set in tenths of a degree. The system ramps up to this temperature and holds that value for a specified amount of time (the hold time).

Gradient Steps

The gradient range is the difference between the lower and upper temperatures in a gradient step. The maximum allowed range is 24°C. The lower temperature is a value between 30.0 and 99.0°C, set in tenths of a degree. The maximum upper temperature is 100°C. The thermal cycler ramps up to the target temperature gradient across the block and holds that temperature for a specified hold time.

Important: CFX Maestro calculates the gradient value. When you enter a value in the gradient calculator's top and bottom fields in CFX Maestro, the software automatically calculates and assigns the temperatures for the remaining fields. When you enter a temperature in any field between the top and bottom field, the software automatically calculates the remaining fields. You cannot manually enter a temperature value in every field.

For more information on how to enter gradient steps in CFX Maestro, refer to the CFX Maestro User Guide.

Table 8. Parameters and ranges for protocol steps

Parameter	Range	Description
Ramp rate	0.1–5°C per sec	Instructs the thermal cycler to ramp to the target temperature at the specified rate in that step. Available only to temperature steps.
Increment	A number from –10.0 to 10.0°C per cycle in tenths of a degree	Instructs the thermal cycler to change the target temperature of a step with each cycle, where a positive number increases the temperature and a negative number decreases the temperature. Available only to temperature steps that are in a GOTO loop.
Extend	A time from –60 to 60 sec per cycle	Instructs the thermal cycler to extend the hold time with each cycle. A positive number increases the hold time and a negative number decreases the hold time. Available to both temperature and gradient steps that are in a GOTO loop.
Beep	(No parameters)	Instructs the thermal cycler to beep to signal that the thermal cycler has reached the target temperature for that step. Available only to temperature steps.
Plate read	(No parameters)	Instructs the thermal cycler to add a plate read to the selected step. Available to both temperature and gradient steps.

Calibrating New Dyes

The CFX Duet instrument is factory calibrated for commonly used fluorophores in white-well and clear-well plates. Table 9 lists the fluorophores and channel for which each instrument is calibrated.

Note: The CFX Duet instrument also includes a channel dedicated to FRET chemistry. This channel does not require calibration for specific dyes.

Important: If you conduct a user-defined calibration of a dye that was factory calibrated, the instrument uses the user-defined calibration instead of the factory calibration. You must use CFX Maestro to conduct a user-defined calibration.

Table 9. Factory calibrated fluorophores and channels

Fluorophores	Channel	Excitation, nm	Detection, nm
FAM, SYBR® Green I	1	450–490	515–530
VIC, HEX, CAL Fluor Gold 540, Cal Fluor Orange 560	2	515–535	560–580
FRET Chemistry (Not Factory-Calibrated)			
Non-factory calibrated color	FRET	450–490	560-580

To calibrate new dyes for the CFX Duet instrument

1. Start CFX Maestro.

2. In the Home window of CFX Maestro, select the CFX Duet instrument in the Detected Instruments pane.

3. Select Tools > Dye Calibration Wizard to open the Dye Calibration wizard.
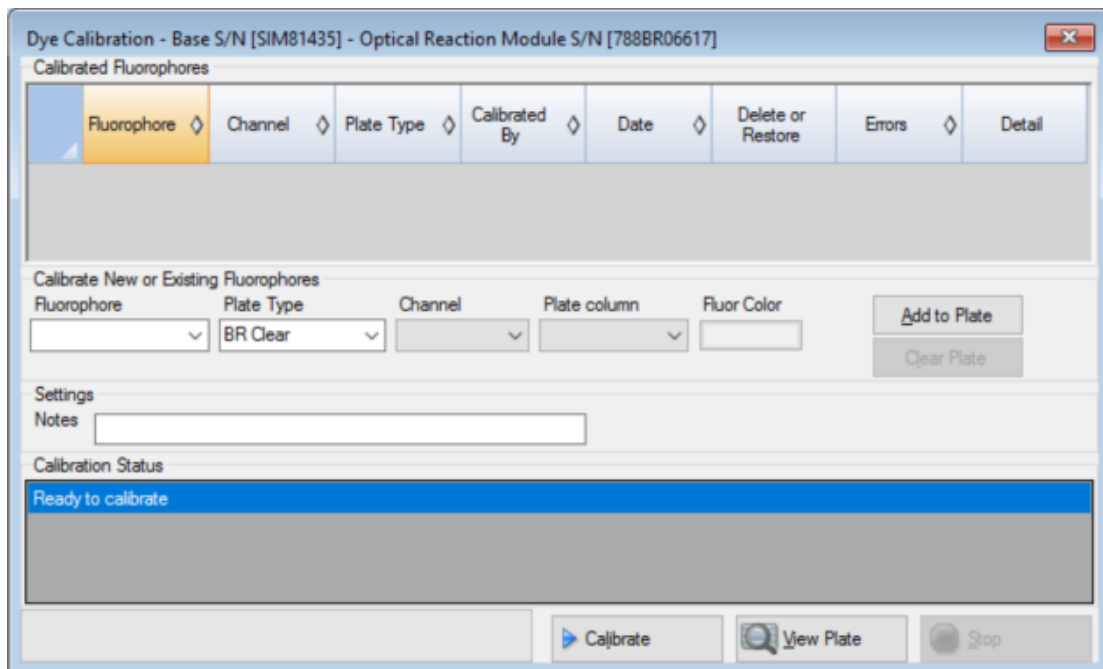
Fluorophores already calibrated for the target instrument appear in the Calibrated Fluorophores table.

4. In the Calibrate New or Existing Fluorophores section, select the fluorophore to calibrate from the dropdown list.

    If the fluorophore name is not included in the list, type its name in the text box to add it to the list.

    Important: Be careful when naming custom calibrated fluorophores. If you create a custom dye calibration for a fluorophore with the same name as a factory calibrated fluorophore, the custom fluorophore (not the factory calibrated fluorophore) will be the one used by the instrument during the runs.

5. Select the plate type for the fluorophore.

    If the plate type is not included in the list, type the name in the text box to add it to the list.

6. Select a channel for the fluorophore.

7. Select a plate column for the fluorophore.

8. (Optional) Type a color to associate with the fluorophore.

9. Click Add to Plate to add the fluorophore.

10. (Optional) Repeat steps to add each fluorophore you plan to calibrate for the plate.

11. When you finish adding fluorophores, click View Plate to open the Pure Dye Plate Display window.

    Use this window as a guide for loading dyes into the plate.

12. Prepare a CFX Duet well plate for dye calibration:

    a. Pipette dye solution into each well, following the pattern shown in the Pure Dye Plate Display.

    b. For each fluorophore, fill four wells with 50 µl of 300 nM dye solution. Notice that at least half the plate contains blank wells.

    c. Seal the plate using the sealing method you will use in your experiment.

13. Place the calibration plate in the block and close the lid.

14. In the Dye Calibration wizard, click Calibrate and then OK to confirm that the plate is in the block.

15. When CFX Maestro completes the calibration run, a dialog box appears. Click Yes to finish calibration and open the Dye Calibration Viewer.

16. Click OK to close the window.

To view Calibrated Dyes:

1. In the Detected Instruments Pane, right-click the CFX Duet instrument and select Properties.

2. In the Instrument Properties Window, click the Calibrated Dyes tab.

	Fluorophore	Channel	Plate Type	Calibrated By	Date	Errors
1	Cal Gold 540	2	BR Clear	Factory	07/20/2021 17:05:42	☐
2	Cal Gold 540	2	BR White	Factory	07/20/2021 16:55:53	☐
3	Cal Orange 560	2	BR Clear	Factory	07/20/2021 17:05:42	☐
4	Cal Orange 560	2	BR White	Factory	07/20/2021 16:55:53	☐
5	FAM	1	BR Clear	Factory	07/20/2021 17:05:42	☐
6	FAM	1	BR White	Factory	07/20/2021 16:55:53	☐
7	HEX	2	BR Clear	Factory	07/20/2021 17:05:42	☐
8	HEX	2	BR White	Factory	07/20/2021 16:55:53	☐
9	SYBR	1	BR Clear	Factory	07/20/2021 17:05:42	☐
10	SYBR	1	BR White	Factory	07/20/2021 16:55:53	☐
11	VIC	2	BR Clear	Factory	07/20/2021 17:05:42	☐
12	VIC	2	BR White	Factory	07/20/2021 16:55:53	☐

To see detailed information about a calibration, click its Info button in the Detail column.

Appendix A Bio-Rad's Real-Time PCR Detection Systems and CFX Maestro Software Catalog Numbers

This appendix lists the catalog numbers for Bio-Rad's CFX Duet Real-Time PCR Detection System, CFX Maestro Software, and accessories.

Table 10. Catalog numbers for Bio-Rad's CFX Duet, accessories, and CFX Maestro Software

Catalog Number	Description
Instruments	
12016265	CFX Duet Real-Time PCR System
CFX Maestro Software and accessories	
12013758	CFX Maestro Software 2.3
12012832	CFX Maestro Software SE
12012833	CFX Maestro Software, Russian Edition
12012834	CFX Maestro Software, Chinese Edition
16007721	USB cable

Appendix B Recommended Plastic Consumables

Plastic Consumables for the CFX Duet System

The CFX Duet instrument accepts low-profile 0.2 ml plates and tubes. Bio-Rad recommends HSP9655 — Hard-Shell low-profile 96-well skirted PCR plates with white shell and white wells for optimal results.

The following additional plastic consumables will fit the system but may need to be validated and optimized for specific workflows:

- MLL9601 — Multiplate low-profile 96-well unskirted PCR plates with clear wells

- MLL9651 — Multiplate low-profile 96-well unskirted PCR plates with white wells

- HSP9601 — Hard-Shell low-profile 96-well skirted PCR plates with white shell and clear wells

- TLS0801 — Low-profile 0.2 ml 8-tube PCR strips without caps, clear

- TLS0851 — Low-profile 0.2 ml 8-tube PCR strips without caps, white

- TCS0803 — Optical flat 8-cap strip, for 0.2 ml PCR tubes and plates

Plate Seals and Plate Sealer

For optimal results, Bio-Rad recommends the following plate seals:

- MSB1001 — Microseal 'B' adhesive seals, optically clear (strong adhesive-based)

- MSC1001 — Microseal 'C' optical seals, optically clear (pressure-activated, adhesive-based)

Appendix C Maintenance and Troubleshooting

This appendix explains how to clean and maintain the CFX Duet Real-Time PCR Detection System and how to troubleshoot problems that you might have. If you need to return the system to Bio-Rad, see Returning the CFX Duet Real-Time PCR Detection System to Bio-Rad.

Cleaning and Maintaining the CFX Duet Real-Time PCR Detection System

The CFX Duet instrument requires little maintenance for proper operation and precise thermal control. However, with long and constant use, the instrument will require some cleaning and other maintenance.

CFX Duet instruments include a sensitive optical shuttle system that moves quickly during data collection and a sample block that must heat and cool quickly. Contamination of these components can interfere with thermal cycling and data collection.

Never allow a reaction to run with an open or leaking sample lid. The reagents could escape and coat the block, inner lid, and optical head in the shuttle system. Excessive dirt can dim the signal, and fluorescence contamination can create excessive background signal. The shuttle system cannot be cleaned except by trained Bio-Rad service engineers.

Avoid contaminating your CFX Duet instrument by following these guidelines:

- Always clean the outside of any containers before placing them in the block.

- Never run a reaction with a seal that is open, loose, punctured, or otherwise damaged because you could contaminate the block, inner lid, and optical system.

- Never run a PCR or real-time PCR reaction with volatile reagents that could explode and contaminate the block, inner lid, and optical system.

- Never run a real-time PCR reaction with volatile reagents that could rupture the sample container.

- Clean the reaction block and inner lid periodically to prevent the buildup of dirt, biohazardous material, or fluorescent solutions (see Table 11 on page 52).

  Important: Never clean or otherwise touch the optical system behind the heater plate holes in the inner lid.

Do not touch these holes

Do not touch anything behind these holes.

■ Clean the outer surface of the CFX Duet instrument on a regular schedule to remove any debris or dirt that might interfere with proper function (see Table 11 on page 52). Clean the instrument to prevent damage to the air intake or reaction bay.

Important: For instructions on handling and cleaning radioactive or biohazardous materials, consult the guidelines for radiation safety and biosafety provided by your institution. These guidelines also include hazardous materials disposal methods.

CFX Duet Real-Time PCR Detection System Cleaning and Maintenance Safety Warnings

When cleaning and maintaining the CFX Duet instrument, always consider and follow the warnings listed in Table 11 that follows.

Table 11. Cleaning and maintenance safety warnings

	Warning
⚠	To prevent electrical shock, always turn off and unplug the instrument from the electrical outlet before cleaning it.
⚠	A thermal cycler operates at temperatures high enough to cause serious burns. Always allow the entire instrument to return to room temperature before cleaning.
⚠ ⚠ ⚠	When handling biohazardous or radioactive samples, adhere to the recommended precautions and guidelines specific to your laboratory and location. These guidelines should include cleaning, monitoring, and disposal methods for the hazardous material(s) you are using. In addition, as identified above, there is a small risk of explosion, or of expulsion of liquids or vapors from the sample container(s). When working with hazardous materials the risk of injury from expelled material is compounded with the risk that the hazardous material themselves could be dispersed in and around the instrument. Users should take appropriate precautions for such a situation.

CFX Duet Real-Time PCR Detection System Maintenance

Table 12 lists the components of the CFX Duet instrument that require maintenance.

Table 12. CFX Duet instrument maintenance

Component	Action
Air vents	Use a soft brush, damp cloth, or vacuum cleaner to remove light dust from the air vents. Remove any heavy dust that is deep in the vents with a vacuum cleaner. **Tip:** Cleaning the vents allows sufficient airflow for precise thermal control during a run.
Outside case of the system	Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and remove the residue completely. **Tip:** Cleaning the outside case prevents corrosion.
Sample block	**Important:** Clean spills immediately to prevent them from drying inside wells. Use disposable plastic pipettes with water (recommended), 95% ethanol, or a 1:100 dilution of bleach in water. Always rinse the wells with water several times to remove all traces of ethanol, bleach, or soap. **Note:** Consider the following when cleaning the sample block: ■ If left in the block wells, bleach, ethanol, or soap could corrode the block and/or destroy tubes and microplates during a run. Always rinse the block thoroughly after cleaning it with any solution other than water. ■ Never clean the sample block with strong alkaline solutions (strong soap, ammonia, or highly concentrated bleach). Never use corrosive or abrasive cleaning solutions. These cleaning agents can damage the block, preventing precise thermal control. ■ Never heat the block with cleaning solution on or in it. Heating the block with cleaning solution damages the block and lid, and can contaminate the optics. If oil is used, the wells must be cleaned thoroughly and often. Use of oil in the wells is **not recommended**. Use a solution of 95% ethanol to clean oil on the sample block. Do not allow oil to build up in the block.

Table 12. CFX Duet instrument maintenance, continued

Component	Action
Inner lid of the sample block	Use a soft cloth and water to remove debris and solutions from the inner lid surface.
	Tip: Cleaning the inner lid improves precise sample heating and cooling.
	Important: Never use abrasive detergents or rough materials that scratch the surface.

Maintaining Sufficient Air Flow

The CFX Duet instrument requires sufficient air flow to heat and cool precisely to the correct target temperature. If the flow of air is blocked, the thermal cycler cannot ramp to the correct temperature in the specified time. This section explains how to test the air flow and how to fix low or warm air flow.

Testing for Sufficient Air Flow

The air flow is sufficient when the instrument heats and cools to the correct target temperatures promptly. Bio-Rad suggests that you test the air flow when you set up the CFX Duet instrument in a new location. You can also measure the air temperature at any time to ensure sufficient air flow.

To determine the presence of sufficient air flow

1. Set up and start the instrument.

2. Adjust the local environment for typical conditions:

   ■ Turn on any nearby equipment, such as fans or other instruments.

   ■ Open any window blinds to reproduce typical conditions during a run.

3. Run a typical PCR protocol for 30 min.

   If more than one instrument is in the area, run a protocol on all instruments at the same time.

   Note: Samples are not required for the test runs. However, you must include an empty microplate or capped strip tubes. The lid does not heat correctly if it touches the sample block.

4. Measure the air temperature at the air intake vents of the instrument.

   If the air intake temperature increases above 31°C, see the following section Fixing Insufficient Air Flow.

Fixing Insufficient Air Flow

If the air temperature near the instrument is above 31°C, make one or more of the following changes to increase the flow of cooler air around the instrument:

■ Adjust air conditioning to lower the ambient air temperature.

■ Move the instrument to another location.

■ Provide more space around the instrument and between adjacent instruments. Arrange instruments so that the warm exhaust air from one instrument does not enter the air intake vents of another.

■ Shield the instrument from heat sources such as radiators, heat-producing instruments, and bright sunlight.

Replacing Fuses

Fuses on the CFX Duet instrument are designed to blow in case of severe power surges or other causes of electrical short. This protects both the user and the instrument from excessive, potentially damaging electric currents. Fuses on the CFX Duet instrument rarely need to be replaced. However, some institutions prefer to replace fuses on a regular basis to maintain uninterrupted operation.

If the instrument does not turn on, first verify that the power cord is plugged in to a functioning power source. Also, verify that the power cord and power source are within the specifications for this instrument.

Important: Do not attempt to replace the power cord on the CFX Duet instrument. Instead, contact Bio-Rad Technical Support.

Finally, verify that the fuses are intact. If the fuses are broken or burned, replace the fuses. This section explains how to view and replace fuses on the CFX Duet instrument.

**Tip:** The instrument utilizes two 10 A, 250 V, 5 x 20 mm, fast blow fuses.

WARNING! To prevent electrical shock, always turn off and unplug the instrument from the electrical outlet before checking the fuses.

To view and replace the fuses

1. Verify that you have turned off the instrument and unplugged the power cord from the back of the instrument.

   Important: You must unplug the power cord from the instrument to open the fuse door. Trying to open the fuse door while the cord is plugged in could damage the door.

2. Using your finger tip, gently pull the black fuse door on the back of the instrument out toward you.
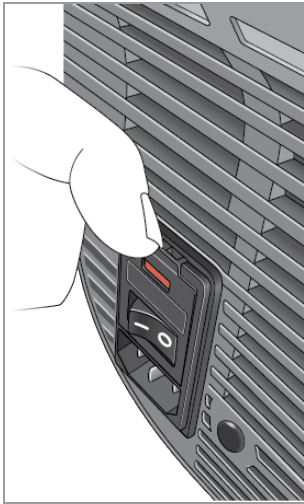
3. Using a small flathead screw driver, gently pry out the red fuse holder until you can grasp it with your fingers.
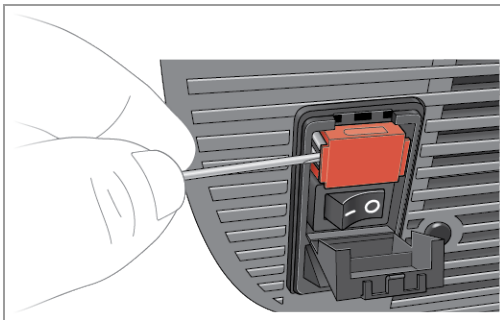
4. When you can firmly grasp the fuse holder, pull it straight out of the instrument.
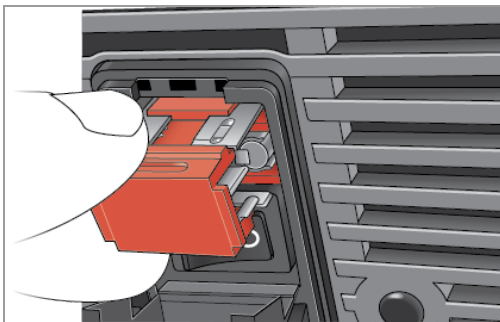
5. Using a finger tip, gently pry the fuse out of the fuse holder.

6. The fuse holder contains two fuses, one on each side. You must inspect both fuses.

A bad fuse displays a break or burned spot in the internal metal filament, or measures open with an Ohm meter. A good fuse has an internal metal filament or measures short (< 1 Ohm). If a fuse is bad or damaged, replace it with a new fuse of the same type and rating.

Note: Some fuses used in the CFX Duet instrument are made from ceramic and cannot be visually inspected. In this case, you must use an Ohm meter to determine whether the fuse is good. Alternatively, you can replace the fuse with a known good one without checking.
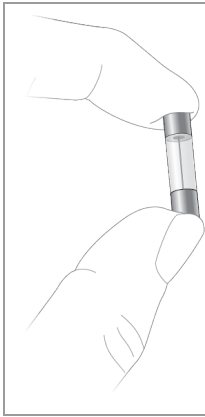
7. Assuming the prongs indicate the front of the fuse holder, insert the back end of a 10 A, 250 V, 5 x 20 mm, fast blow fuse into the middle bracket. Ensure the front end of the fuse faces the prongs. For example:
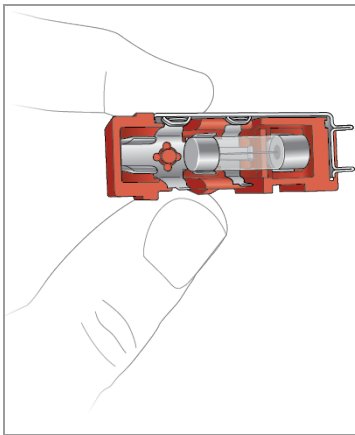
8. With the flat red square on the fuse holder facing upward, insert the fuse holder into the instrument and firmly press it into position.
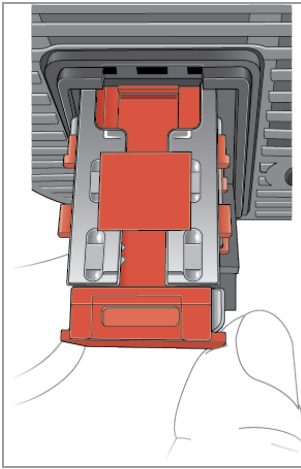
9. Close the fuse door, insert the power cord, and turn on the instrument.

⚠️ **Caution:** If the CFX Duet instrument repeatedly blows one or more fuses, there could be an internal problem with the instrument. Contact Bio-Rad Technical Support for assistance in determining whether it is safe to replace the fuses again or if the instrument should be repaired.

# Upgrading Software and Firmware on CFX Duet Real-Time PCR Systems

You can only upgrade CFX Duet instrument firmware and CFX Maestro software in CFX Maestro.

Note: Depending on the type of upgrade, this process can take several minutes to complete.

Updating CFX Maestro Software and CFX Duet Real-Time PCR Detection System Firmware

When connected to the internet, CFX Maestro automatically checks for both CFX Maestro and CFX Duet updates each time it starts. CFX Maestro can automatically perform the CFX Duet update.

When updates are available, the Software and Instruments Updates dialog box automatically appears.
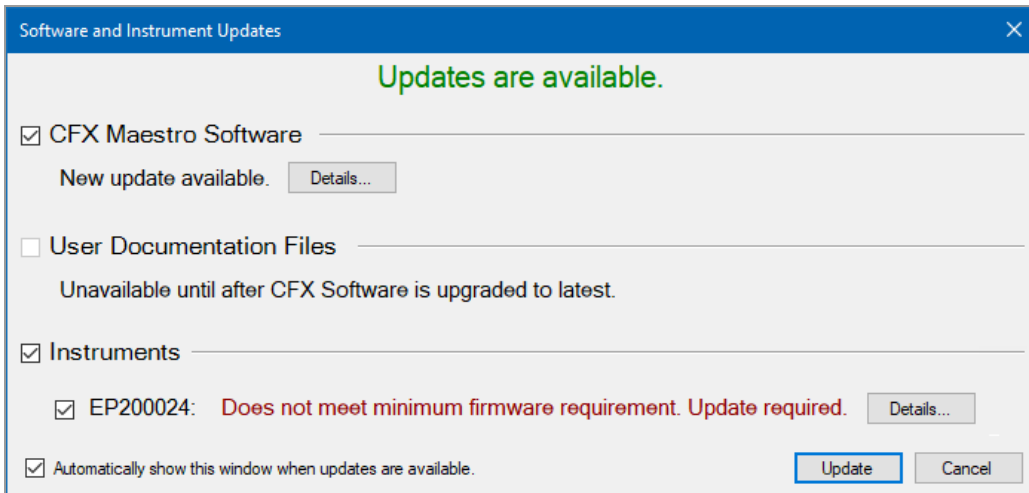
Important: CFX Duet instruments requiring an update appear with a red alert. You must perform the required update in order to run experiments on that instrument.

When both software and system updates are performed, the software is updated first. In order to download the latest updates, the computer running CFX Maestro instrument must be connected to the internet. Close all data analysis windows and ensure that all instruments are idle before beginning the update.

When the software update completes, CFX Maestro restarts and then begins the CFX Duet updates. CFX Maestro determines whether the instrument software is compatible with the version of CFX Maestro that is installed. If the instrument software is not compatible, this update begins automatically. No internet connection is required for this operation.

Note: You must restart the instrument after the update completes.

To perform software and system updates

▶ In the Software and Instrument Updates dialog box, do one of the following:

■ If either a software or system update is available, select the appropriate checkbox and click Update.

■ If both software and instrument updates are available, select both checkboxes and click Update.

Tip: In this case, updating the software requires an instrument update for any connected instruments to ensure proper communication.

To hide the Software and Instrument Updates dialog box alert

▶ To hide the Updates dialog box when new updates are available, clear the Automatically show this window when updates are available checkbox.

Important: If an attached instrument requires an update, the Software and Instrument Updates dialog box automatically appears even if this checkbox is cleared. You must perform the requisite update in order to run experiments on that instrument.

To display the Updates dialog box, on the Home window select Help > Check for Updates and select the Notify when updates are available checkbox.

Shutting Down the CFX Duet Real-Time PCR Detection System

Follow these instructions to safely and completely shut down the CFX Duet instrument.

To shut down the CFX Duet instrument

1. Ensure that no protocol is running and the instrument is no longer in use.

2. In the CFX Maestro Detected Instruments pane, select the CFX Duet instrument.

3. Click Open Lid.

4. Remove the sample plate located on the block of the CFX Duet instrument.

5. Click Close Lid.

6. Press the power switch on the back panel of the instrument to power down the system.

Returning the CFX Duet Real-Time PCR Detection System to Bio-Rad

Important: Should you need to return your CFX Duet instrument to Bio-Rad, your Bio-Rad Technical Support specialist will provide instructions for decontaminating, packaging, and shipping the instrument. Before you return the instrument, you must install the shipping plate and shipping screw. This section explains these tasks.

Note: Locate the shipping screw and shipping plate that you saved when you installed the system. You will need these items to properly package the system. Bio-Rad will send you the packaging material required to safely return the system.

Important: Before you begin, ensure that you have completely backed up all data files to a shared network drive or a USB drive.

Installing the Shipping Plate and Screw

To install the shipping plate and screw

1. Start the CFX Duet instrument and log in to CFX Maestro.

2. In CFX Maestro, transfer all data files from the CFX Duet instrument to a computer running CFX Maestro. For instructions on how to transfer user data, refer to the CFX Maestro User Guide.

3. To install the shipping screw:

   a. Right-click on the instrument in the Detected Instruments pane of CFX Maestro and select Properties.

   b. Click the Shipping Screw tab.

   c. Click the Open Lid button.

   d. Place the shipping plate on the CFX Duet instrument sample block.

   e. Click the Close Lid button.

   f. Click the Lock Lid button.

   g. Confirm that the CFX Duet instrument shipping plate is installed, and click the Yes button in the confirmation dialog.

4.   Remove the beige plug (store it in a safe place) and then install the shipping screw, turning it clockwise until it is securely in place.

5.   Click the Screw Installed button to confirm that the shipping screw is installed.
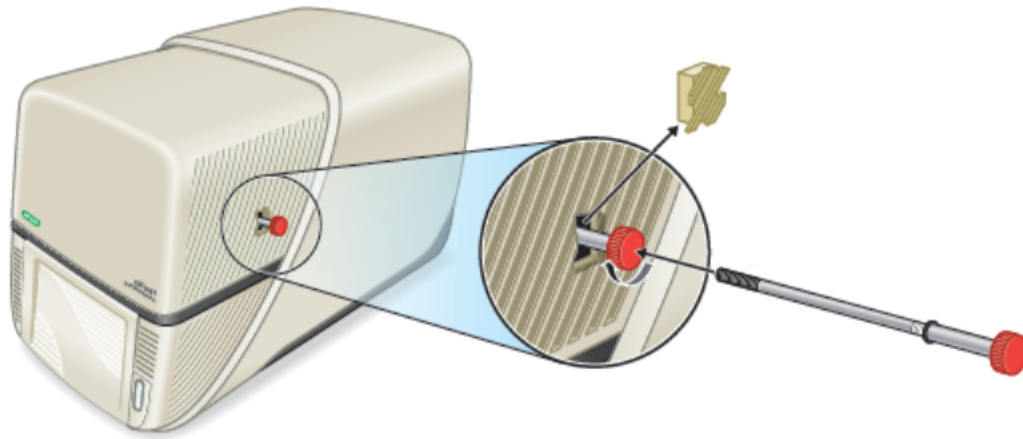
6.   Log out of CFX Maestro and then shut down the CFX Duet instrument.

7.   Package and ship the system to Bio-Rad according to the instructions provided by Bio-Rad.

Appendix D Bio-Rad Free and Open-Source Notices for PCR Products

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Bio-Rad source code site:

https://github.com/bio-rad-lsg-open-source/Mqtt-4.3.0.0

External source code site:

https://github.com/eclipse/paho.mqtt.m2mqtt

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February 1, 2011

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Appendix E References

1. Breslauer KJ et al. (1986). Predicting DNA duplex stability from the base sequence. Proc Natl Acad Sci USA 83, 3,746–3,750.

2. Sugimoto N et al. (1996). Improved thermodynamic parameters and helix initiation factor to predict stability of DNA duplexes. Nucleic Acids Res 24, 4,501–4,505.

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