STANDARD OPERATING PROCEDURE

Title	CFX96™: programming, starting a run and analyzing data
Short title	CFX96™

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1   Purpose

This SOP describes how to use the real time CFX96™ platform of BIO-RAD PCR as PCR detection system. It focuses on how to set up and program the device, as well as how to start a run and perform data analysis. This SOP is specifically meant for laboratory personal trained to set up and run PCR reactions on BIO-RAD CFX96 system.

2   Scope

This Standard Operating Procedure (SOP) applies to all study team members involved in performing real-time polymerase chain reaction.

3   Abbreviations and definitions

Abbreviations and definitions included in alphabetical order

3.1   Abbreviations

Abs.	absolute
BNITM	Bernhard Nocht Institute for Tropical Medicine
Hz	Hertz
kVA	Kilovolt ampere
N/A	Not applicable
SOP	Standard Operating Procedure
TEs	thermal electric modules
VAC	Volt Alternating Current
VIR	Department of Virology

3.2   Definitions

SOP	A detailed, written instruction to archive uniformity of the performance of a specific function.

4   Responsibilities

Role/Title	Responsibilities/Tasks
Head of Laboratory	Release valid version of the SOP for use in the laboratory.
Designated senior laboratory staff	Take care of the regular review process for the SOP and supervise SOP and process specific trainings and the corresponding documentation.
All laboratory staff	Use and maintenance of the CFX96™ in accordance to this SOP and the user manual.

5   General description

The BIO-RAD CFX96™ PCR machine is a six-channeled system that combines advanced optical technology with precise thermal control to deliver sensitive, reliable detection. Runs can be set up quickly and amplification traces are monitored in real time on the integrated LCD touchscreen. The system has a stand-alone mode, so that it does not need to be connected to a laptop.

The rate and efficiency of PCR depend critically on the precision of the temperature steps. To obtain reliable, consistent results, all sample wells must maintain proper temperature throughout each incubation step. The CFX96™ system uses six independently controlled thermal electric modules (TEs), the heating and cooling elements of the thermal cycler, to maintain tight temperature uniformity at all points during the run — even while ramping.

A key component of overall protocol run time is the time to reach target temperature, which is determined by the average ramp rate and the time for the sample block to reach thermal uniformity. Maximum ramp rate is less important, because it can fluctuate significantly during the ramp. The CFX96™ system's temperature control produces high average ramp rates and tight uniformity during ramping, to yield fast time to target temperature and faster protocol run times. The CFX96™ system's solid-state optical technology (six filtered LEDs and six filtered photodiodes) provides sensitive detection for precise quantitation and target discrimination. Scanning just above the sample plate, the CFX96™ optics shuttle individually illuminates and detects fluorescence from each well with high sensitivity and no cross talk.

The optical system automatically collects data from all wells during data acquisition, so you can enter or edit well information on your own schedule.

The CFX96™ system can discriminate up to five targets in a single reaction well. The optical filter sets are designed to maximize fluorescence detection for specific dyes in specific channels. At every position and with every scan, the CFX96™ optics shuttle is reproducibly centered above each well, so the light path is always optimal and there is no need to sacrifice data collection in one of the channels to normalize to a passive reference.

6   Device requirements

- Power source input power: 100 - 240 VAC, 50 - 60 Hz
- Indoor use: ambient temperature of 15 – 31 °C Relative humidity maximum of 80 %, noncondensing.
- USB cable. If the system is going to be controlled by a computer via a USB cable, the provided cable from Bio-Rad is sufficiently shielded for use.
- The CFX96™ real-time PCR detection system should be installed on a clean, dry, level surface with sufficient cool airflow to run properly.
- The CFX96™ system or CFX384™ system can be run in two modes: stand-alone or software-controlled. If you are running the system under software-controlled mode, make sure there is sufficient space for a computer during setup.

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7 Material and equipment

7.1 General
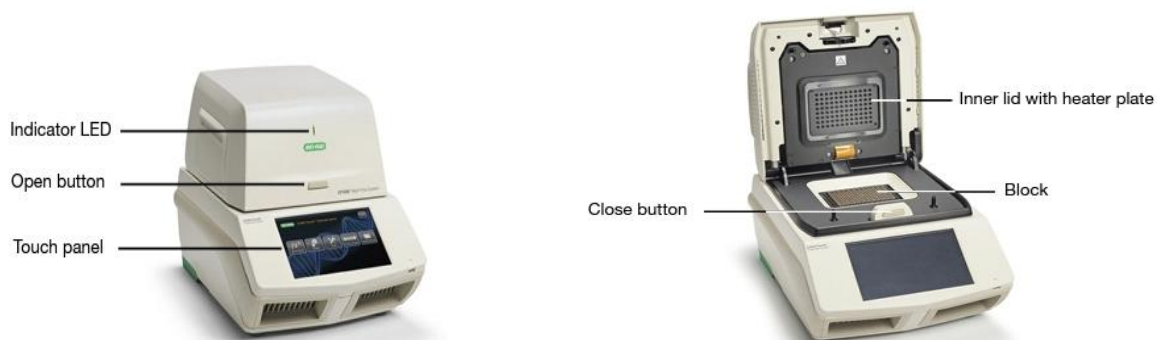
BIO-RAD CFX96™ detection system, installation guide and user manual
- UPS 2.2kVA to provide stabilized electricity and battery and USB cable
- Laptop connected to the BIO-RAD CFX96™ with CFX96™ software installed
- Strip tubes and caps or plates and sealing foils
- Disposable gloves
- Loading/cooling block

7.2 Maintenance

Lint-free cloth
Brush
Ethanol, abs.

7.3 Storage

N/A

8 Safety

When operating the CFX96™, always wear disposable gown and gloves.
Refer to the CFX manual for detailed safety advisories.

9 Procedural description

9.1 Start-up process

9.1.1 Functional check

Make sure you have access to UPS to ensure proper power supply during the run and make sure the device and laptop are connected to it. Switch on the laptop, then switch on the CFX. Open the CFX Manager ™ Software by double clicking the icon on the desktop. Make sure, that the software is connected to the machine – you will find the serial number of the instrument on the upper left side of the window.

9.1.2 Calibration

N/A

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9.1.3   Validation
N/A

9.2   Loading the CFX96

- Make sure that the PCR reactions are well closed and spun down.
- Click the „Open Lid" button located on software's Start Run tab or press the lid button on the front of the system to start opening the motorized lid.
- Place the 0.2 ml microplate or tube strips with sealed lids in the block.
- Close the lid.
  Be sure that nothing is blocking 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 closing lid.
- Always balance the tube strips or cut microplates in the wells.
  For example, if you run one tube strip on the left side of the block, run an empty tube strip (with caps) on the right side of the block to balance the pressure applied by the heated lid.
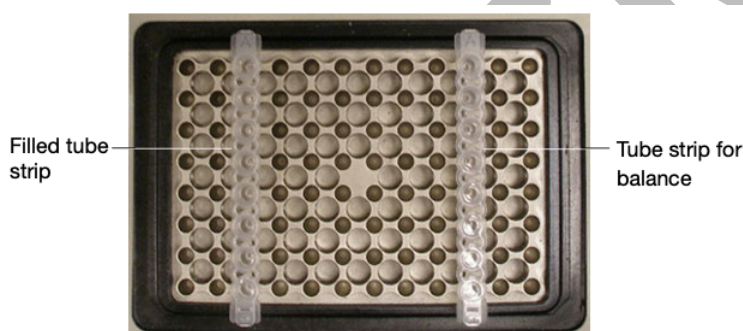
9.3   Run setup and starting

The Run Setup window provides quick access to the files and settings needed to set up and start a run. To open the Run Setup window, perform one of these options:
- Click "User-defined" in the Run Setup tab of the Startup Wizard
- Click "User-defined Run Setup" in the main software toolbar
- Select "File" > "New" > "User-defined Run" in the main software menu bar

The Run Setup window includes three tabs: **Protocol, Plate** and **Start Run.**

Protocol:
Click the Protocol tab to select an existing protocol to run, edit or create a new protocol in the Protocol Editor window.
- Click "Create New" button to create a new protocol.
- Click Select Existing button to open a browser window to select and load an existing protocol file (.prcl extension) into the Protocol tab.
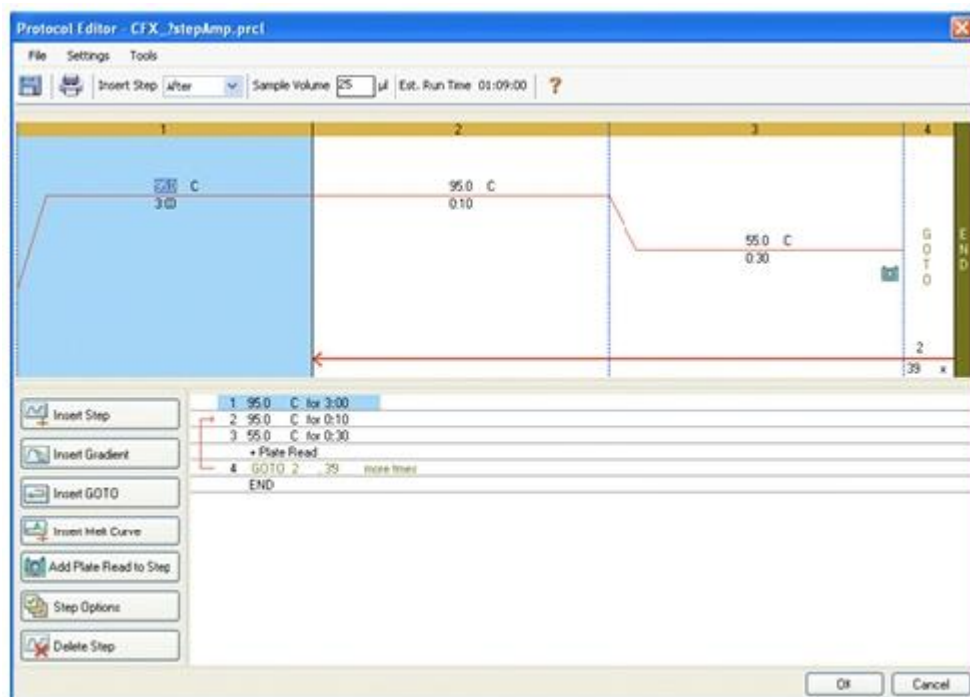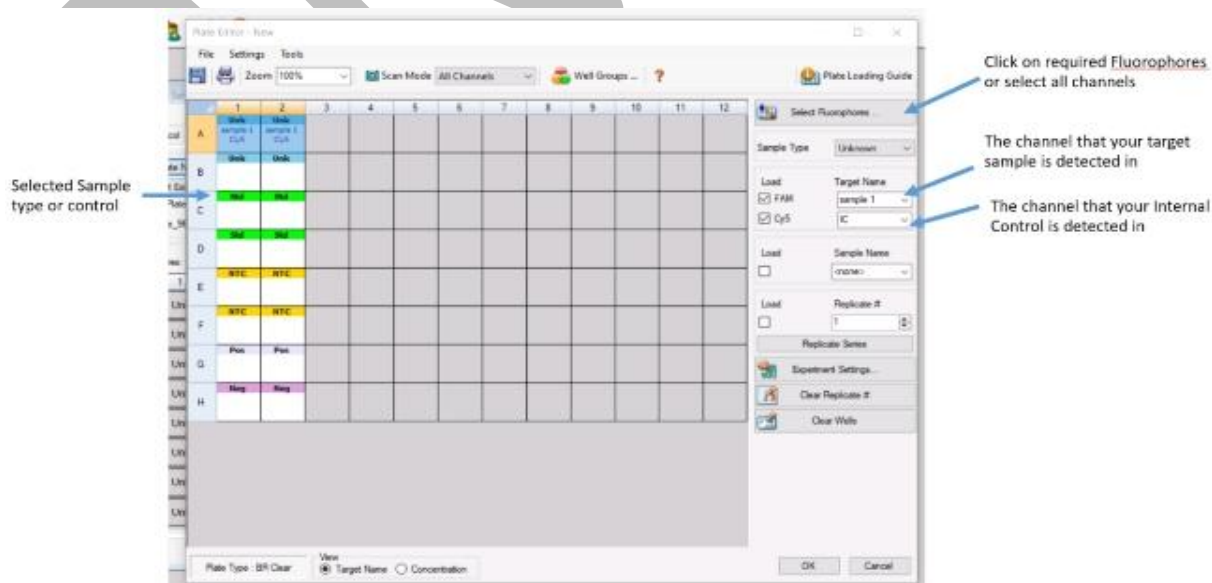
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Plate:

The Plate tab shows a preview of the selected plate file loaded in Run Setup. The plate file (.pltd extension) contains a description of the contents of each well, the scan mode, and the plate type. The CFX Manager ™ Software uses these descriptions for data collection and analysis. Select one of the following options to select an existing plate, create a new plate, or edit the currently selected plate:

- Click "Create New" button - opens the Plate Editor to create a new plate.
- Select "Existing" button - opens a browser window to select and load an existing plate file into the Plate tab.

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- Choose the plate file and click "Edit Existing" to configure current run using the PLATE PLAN.
- Configure the plate plan as follows:

  - Assign Sample type (Unknown, Standard, Positive control, etc.) as in your plate plan.
  - Select Fluorophores to be screened (To open the Select Fluorophores window, click the Select Fluorophores button on the right side of the Plate Editor. Click Load to assign selected dyes (Fluorophores) into all selected wells).
  - Select 'Unknown' samples and set up Replicate series if running replicates.
  - Select plate type: Click on Settings → Plate Type → BR Clear or BR White (according to the used consumables).
  - Click 'Apply' and then Click OK.

Start run:
- Click the Start Run tab to check the run settings, select the instrument block, and plate plan combined together.
- Double-check that the plate /tube strips are loaded as indicated on your PCR working sheet
- A prompt to save will pop up.
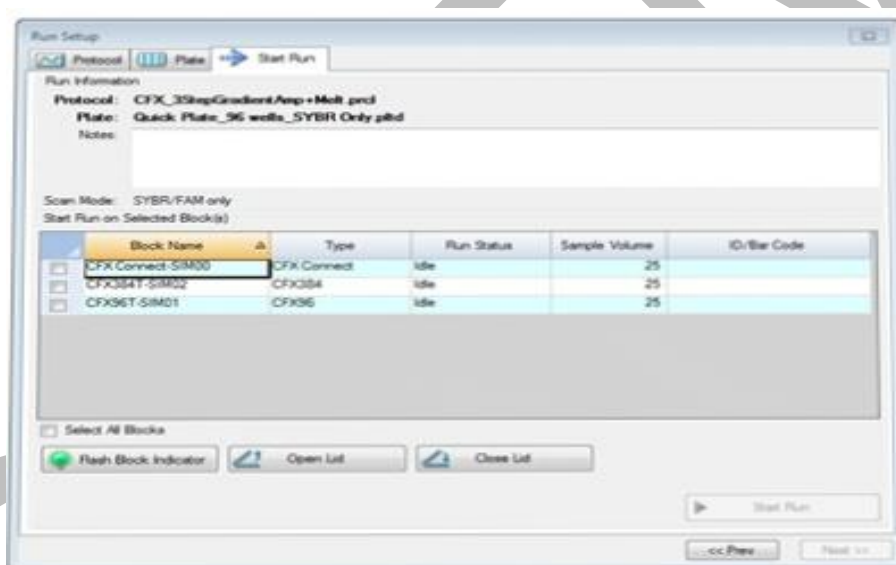
- Save the run in the provided (study-related) folder.
- The run will begin automatically once the run name is saved.
- The remaining run time will be shown in the window below.

9.4   Run analysis

At the end of the run, the analysis page will pop up in the format below with four sub-windows in one. The sub-windows include **amplification chart, standard curve, well selector and spreadsheet.**

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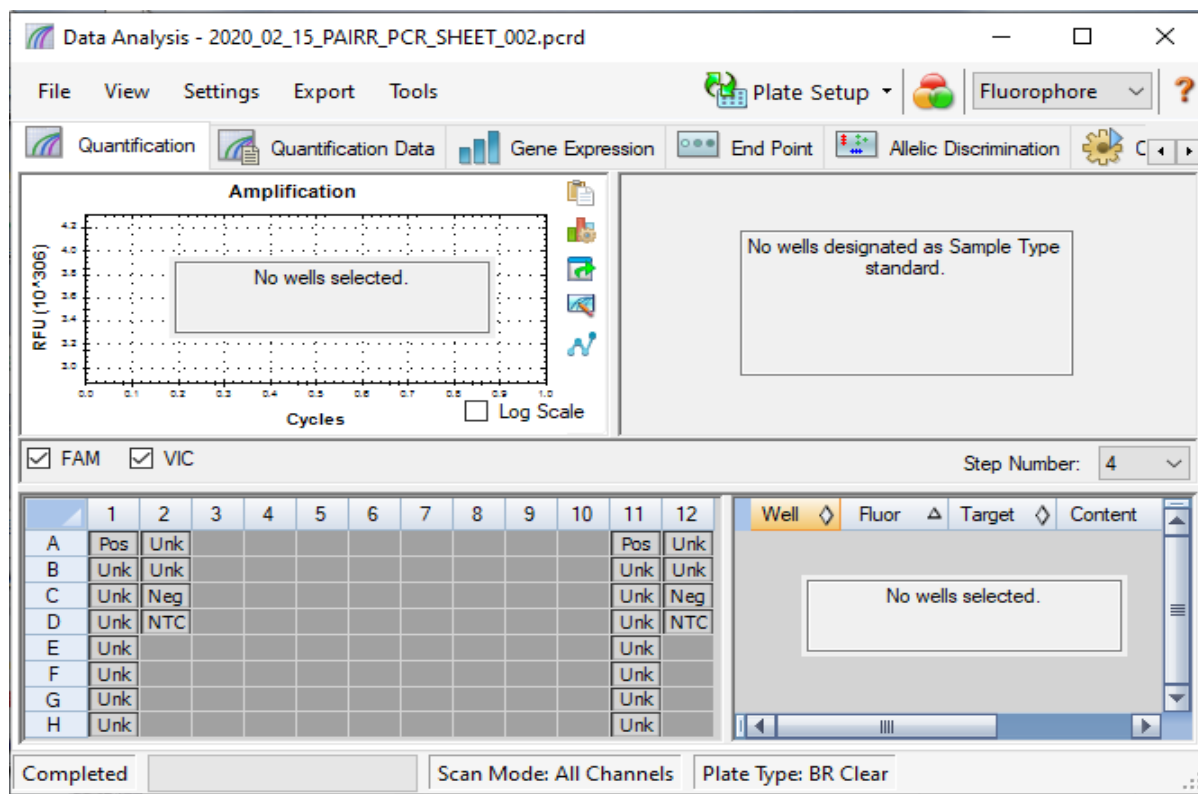
9.4.1 Baseline threshold setting

Set or adjust the threshold for all required channels, one by one:

- In the single threshold mode, select the fluorophore in the fluorophore selector of the quantification tab by clicking the boxes next to the fluorophore name located under the Amplification chart.
- Select Settings > Baseline Threshold in the menu bar to open the Baseline Threshold window.
- Adjust the threshold for the chosen fluorophore: click User Defined and enter the desired threshold value.
- Click OK to confirm and close the window.

NOTE: The threshold should be set to a value, that is crosses the amplification curves at beginning of their exponential phase, just above the background fluorescence.

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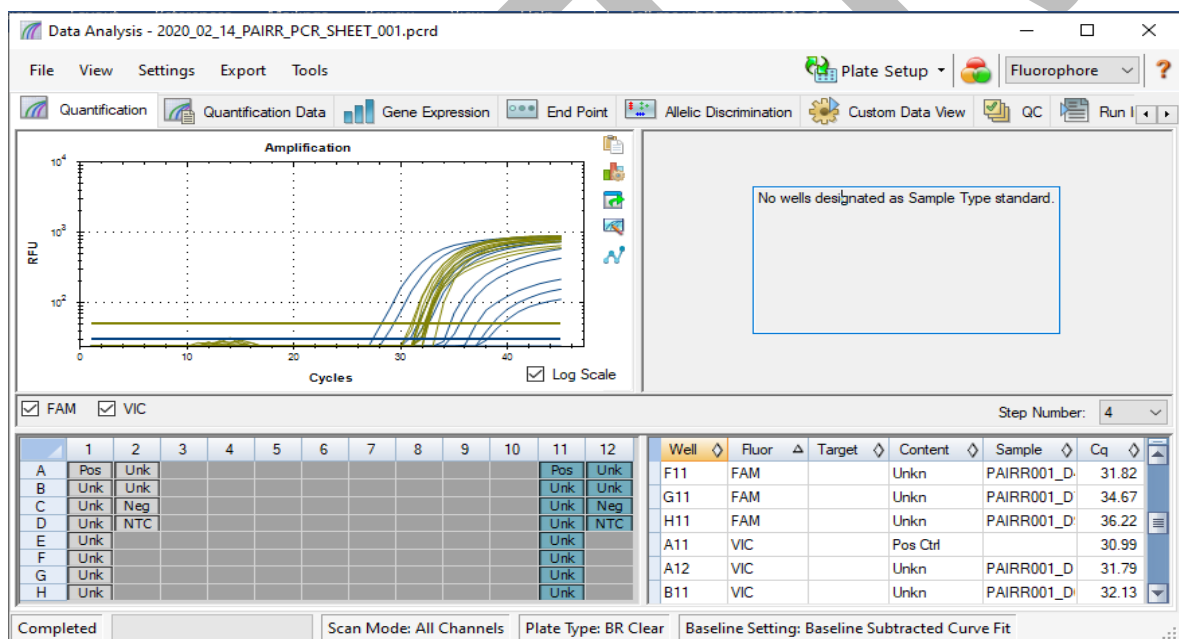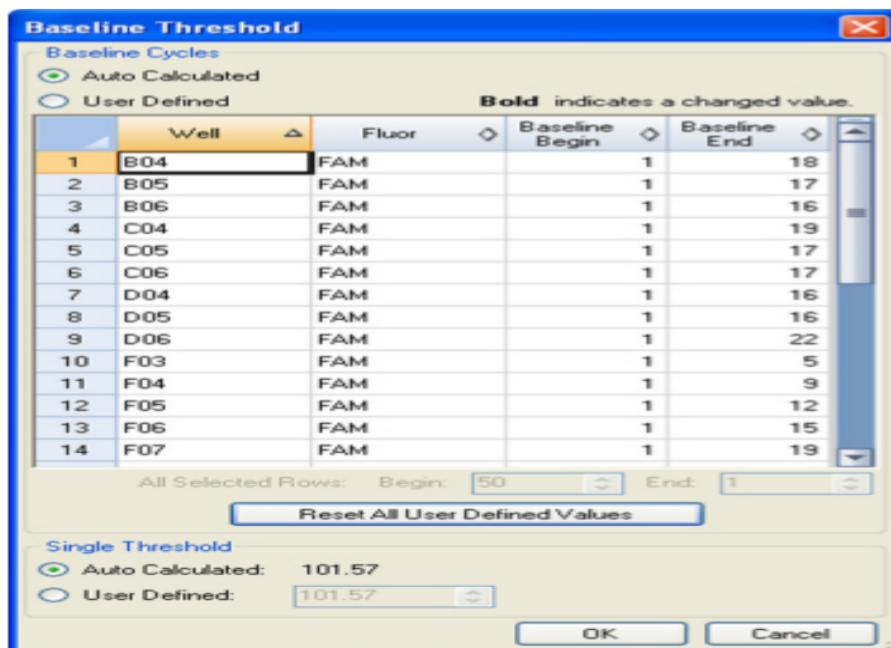
9.4.2 Analysis of controls – run and batch validity

- The **Positive Control (POS)** should be positive and show a Cq value within the expected/ defined range for both, the target channel and the Internal Control channel.
- The **Negative PCR Control (NTC)** should be negative in the target channel(s) and positive, within the expected range, for the Internal Control channel.

When the two above criteria are fulfilled, the **PCR run is valid.**

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- The **Negative Extraction Control (NEG EXT)** should be negative in the target channel(s) and positive, within the expected range, for the Internal Control channel.

When the Negative Extraction Control's results is/are as described above, **the batch(es) of extraction is/are valid**, too. Proceed to sample analysis.

If the controls show different results, the run and/or batch(es) of extraction are not valid, proceed to troubleshooting (see 12. Troubleshooting) and do not validate the samples.

9.4.3 Analysis of samples

- Once the validity of the controls is confirmed, the sample results can be checked, one by one.
- Display them always in parallel with:
  - the Positive Control, so see a good amplification and compare the fluorescence height and
  - the Negative Extraction Control, to compare the Cq value of the Internal Control.
- Analyze each single sample in all channels that are included in the PCR assay used and not down the values on the results sheet.

10 Waste management

After a PCR run, wear gloves to dispose the used consumables and plasticware which may contain hazardous chemicals in designated waste bags. Waste bag will be sealed when full, collected and disposed of properly according to local safety regulations.

11 Maintenance

11.1 Internal

The CFX96™ system includes a sensitive optical shuttle system that moves quickly during data collection and a sample block that must heat and cool very fast. Contamination of these components can interfere with thermal cycling and data collection.

WARNING! 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 the CFX96™ instrument by following these suggestions:

- 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.
- Clean the block and inner lid periodically to prevent the buildup of dirt, biohazardous material, or fluorescent solutions.
- Never clean or otherwise touch the optical system behind the heater plate holes are in the inner lid.

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- Clean the outer lid and C1000 base on a regular schedule.
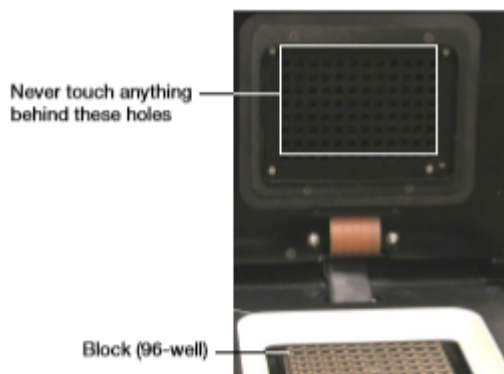
11.1.1 Weekly Maintenance
N/A

11.1.2 Monthly Maintenance

Cleaning the Optical Reaction Module:
The block of the optical reaction module should be cleaned, along with the thermal cycler base, on a regular schedule to remove any debris or dirt that might interfere with proper function. Clean as soon as you discover debris and spilled liquids with a soft, lint-free cloth that is dampened with water. Cleaning the instrument allows precise instrument function.

- Never use cleaning solutions that are corrosive to aluminum. Avoid scratching the surface of the C1000 reaction module bay. Scratches and damage to this surface interfere with precise thermal control.
- Never pour water or other solutions into the C1000 reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.
- Clean the outer lid and C1000 Touch base or CFX Connect base on a regular schedule
- To prevent electrical shock, always remove the reaction module from the thermal cycler base or unplug the base before cleaning the instrument.

Cleaning the outer surface:
- Clean the outer surface. Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution and then rinse the surface with a damp cloth. Cleaning the cover will prevent corrosion

Cleaning the cooling fins:
- Remove dust with a soft brush or damp cloth. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Use water and a soft, lint-free cloth to remove debris that is stuck to the fins. Avoid scratching the surface. If needed, use a mild soap solution and rinse well to remove residue completely. Cleaning the fins improves precise sample heating and cooling.
- Use of oil in the wells is not recommended. If oil is used, the wells must be cleaned thoroughly and often. Remove the oil when it is discolored or contains dirt. Use a solution of 95% ethanol to clean oil. Do not allow oil to build up in the block.
- Clean the wells in the block. Clean spills immediately to prevent them from drying. Use disposable plastic pipets with water (recommended), 95 % ethanol, or a 1:100 dilution of

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bleach in water. Also use a soft, lint-free cloth or paper towel and water to clean the block. Always rinse the wells with water several times to remove all traces of cleaning reagents.

11.1.3 Yearly maintenance

N/A

NOTE: Never clean the block with strong alkaline solutions (strong soap, ammonia, or high-concentration bleach). Never use corrosive or abrasive cleaning solutions. These cleaning agents can damage the block and prevent precise thermal control.

Document all maintenance activities on the maintenance form.

11.2 External

N/A

12 Troubleshooting

12.1 Software communication problems

Typically, software and instrument communication problems can be resolved by restarting your computer and the system. Be sure to save any work in progress before restarting.

Check that your computer has sufficient RAM and free hard drive space. The minimum RAM is 2 GB and the minimum hard drive space is 20 GB.

12.2 Power failure

In a power failure, the instrument and computer will shut down. If the power failure is short, the instrument will resume running a protocol but the Application Log will note the power failure. Depending on the computer settings and the length of time the power is off, the instrument and software attempt to continue running depending on the protocol step:

- If the protocol is in a step with no plate read, then the protocol continues running as soon as the instrument gets power again.
- If the protocol is in a step with a plate read, then the instrument waits for the software to restart and resume communication to collect the data. In this situation, the protocol continues only if the software is not shut down by the computer. When the computer and software start up again, the protocol continues.

If you want to open a locked motorized lid on a reaction module to remove your samples during a power failure, see CFX96 manual.

13 References

Breslauer KJ, et al. (1986). Predicting DNA duplex stability from the base sequence. Proc Nat Acad Sci 83, 3746–50.
Hellemans J, et al. (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data, Genome Biol, 8, R19.

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Livak JL, et al. (1995). Towards fully automated genome-wide polymorphism screening. Nature Genetics 9, 341–342.

Pfaffl MW. (2001). A new mathematical model for relative quantification in real-time RT- PCR. Nucleic Acids Research 29(9), 2002–2007.

Vandesompele J, et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biology 3(7), 1– 12.

14 Associated Documents

14.1 Tools associated with this SOP

Maintenance form
PCR results form

14.2 Other documents associated with this SOP

CFX96™ manual

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15 Document History

Review date	Version number	Brief description of changes
N/A	V01	First release