

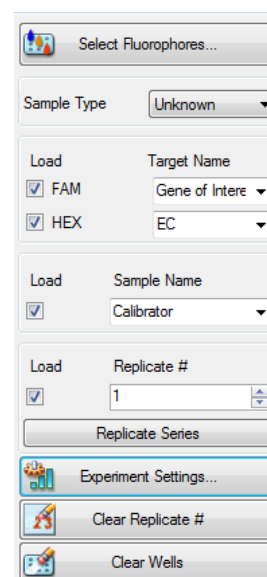
QuickGuide: RealFast™ CNV on BioRad CFX96™

Setup for Relative Quantitation Assays:

- Open the BioRad CFX Manager.
- In the Startup Wizard select instrument **CFX96™** and run type **User-defined**.
- In the Run Setup select **Create New** within the **Protocol Tab**. The **Protocol Editor** opens.
 - Select a sample volume of 20µl and setup the **PCR program**: 10min at 95°C followed by 40 times 15s at 95°C and 60s at 60°C. Make sure that the **Plate Read** is added to the 60°C step. Press **OK** and save the protocol file. Press **Next**.

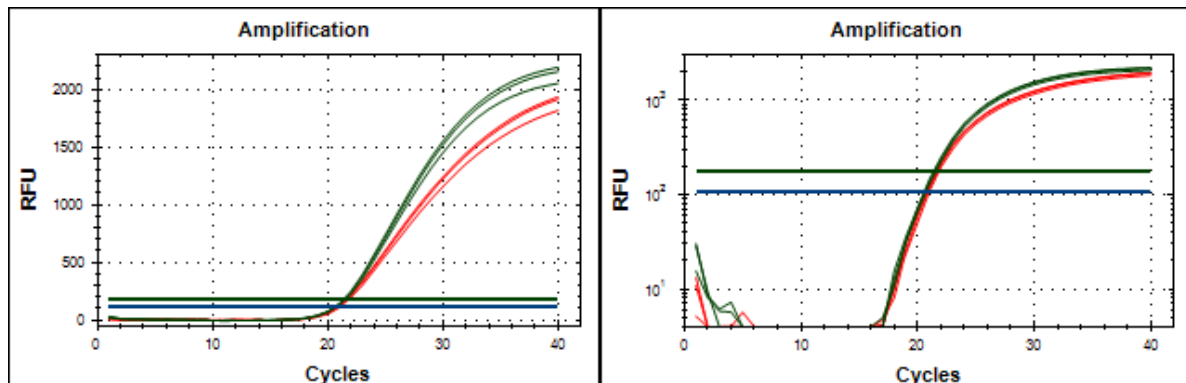
1	95,0	C for 10:00
2	95,0	C for 0:15
3	60,0	C for 1:00
		+ Plate Read
4	GOTO 2	,39 more times
		END

- In the tab called **Plate** select **Create New**. The **Plate Editor** opens.
 - Select **Settings > Plate Type** and choose the correct type of plate.
 - Select scan mode **All Channels**.
 - Click **Select Fluorophores** and select FAM and HEX.
 - Select wells by clicking in the well selector and choose the sample type (**NTC** or **Unknown**); a minimal setup should contain triplicates of a negative control template, the calibrator and an unknown sample.
 - Click **Load** check boxes to load fluorophores **FAM** and **HEX**
 - Type target names
(FAM: gene of interest; HEX: EC=endogenous control)
and press **Enter**. The name is now available from the drop down menu.
 - Type sample name and press **Enter**.
 - Click check box to load **Replicate** number.
 - Open **Experiment Settings** and select the endogenous control **EC** as **Reference** in the **Targets** tab. In the **Samples** tab select the supplied calibrator sample as **Control**. Press **OK**.
 - Choose **Well Groups** in case you are running several assays at the same time
 - Press **OK** and save the plate file.
- Press **Next**, load your PCR tubes and start the run.





Analysis of Relative Quantitation Assays:

- Open the data file: **File > Open > Data File**
- Within the **Quantification** tab select your samples and click the check box for **HEX** in the amplification chart.
 - Right-click on the threshold line and select **Show Threshold Values**.
 - Set the threshold according to the settings in the Assay Description: **Settings > Baseline Threshold > Auto Calculated**
 - Follow the same principle and review the threshold for FAM.
- Select both fluorophores and review the results by clicking on individual wells in the well selector.



Example: Amplification Plot of the Calibrator sample – linear and log graph type (red: gene of interest, green: EC)

- Click on the **Gene Expression** tab. In the **Bar Chart** select a Mode from the drop down menu to the right of the chart. Select **Normalized Expression ($\Delta\Delta Cq$), Relative To Control** (Graph Data) and **Calibrator** (Control Sample). Review **Experiments Settings** for correct assignment of reference (EC) and control (ViennaLab's calibrator). For details right-click on the table in the **Bar Chart** and select **Show Details**.

- In the Results Chart you can find the **Relative Normalized Expression** column. The ratio for the **Calibrator** (Control Sample) is set to „1“ and values for your samples are relative to the **Calibrator**. Refer to the product description for interpretation of your results.

- To open a report, select **Tools > Reports** and adjust the report according to your needs.