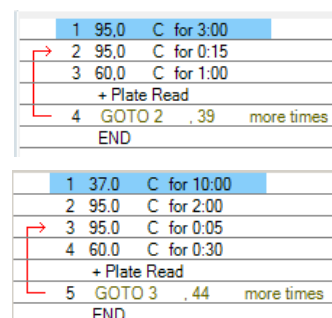


QuickGuide: RealFast™ Variant Detection on Bio-Rad CFX96

Setup for Variant Detection and Onco RealFast™ Assays:

- Open the Bio-Rad CFX Maestro software (QuickGuide is based on version 2.2).
- 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 20 µl and set up the PCR program according to the Instructions for Use of your RealFast™ Assay:
 - For Variant Detection RealFast™ Assays (e.g. HLA-B27) only:
3 min at 95°C followed by 40 times 15 sec at 95°C and 60 sec at 60°C.
 - For Onco RealFast™ Assays (e.g. EGFR T790M) only:
10 min at 37°C followed by 2 min at 95°C followed by 45 times 5 sec at 95°C and 30 sec at 60°C.
 - Press „OK“ and save the protocol file. Press „Next“.



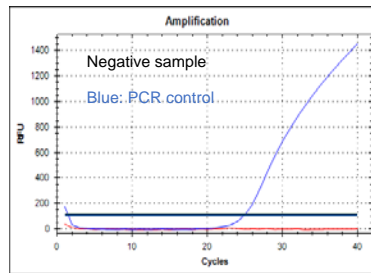
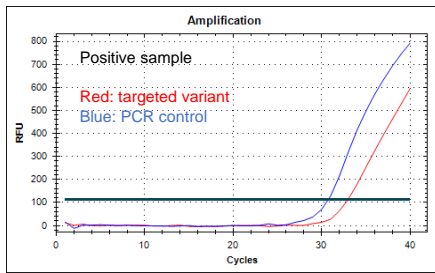
1	95,0	C for 3:00
2	95,0	C for 0:15
3	60,0	C for 1:00
+ Plate Read		
4	GOTO 2	,39 more times
END		

1	37,0	C for 10:00
2	95,0	C for 2:00
3	95,0	C for 0:05
4	60,0	C for 0:30
+ Plate Read		
5	GOTO 3	,44 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** for the No Template Control and **Unknown** for your samples, as well as for Controls.
 - In the field **Target Names** check boxes to load fluorophores **FAM** and **HEX**.
 - Type target names (FAM = Variant; HEX = PCR control) and sample name and press **Enter**.
 - Click check box to load **Replicate** number
 - Define **Well Groups** in case you are running several assays at the same time
 - Press **OK** and save the plate file.
- Press **Next** and load your PCR tubes; start the run.

Analysis of Variant Detection and Onco RealFast™ Assays:

- Open the data file: **File > Open > Data File**.
- Select the **Quantification** tab
 - Select the well group (top right) in case you were running several assays at the same time.
 - Set the threshold according to the Instructions for Use of the respective RealFast™ Assay (e.g. set the threshold value for the FAM channel just above the background fluorescent signal generated by the HLA-B27 Negative Control. Set the threshold value for the HEX channel at the onset of the exponential phase of the amplification curve).
 - Review your samples individually. Samples positive for the targeted variant (e.g. HLA-B27), as well as the Positive Control will show a strong fluorescent signal in both, the **FAM** and the **HEX** channels. Samples negative for the targeted variant, as well as the Negative Control will show a signal in the **HEX** channel only (PCR control).



» **Note:** For **Onco RealFast™** Assays (e.g. EGFR T790M), it is **mandatory** to do the analysis according to the section *Data Analysis / Interpretation of Results in the Instructions for Use*. «

- To open a report, select **Tools > Reports** or click the **Reports** button on the toolbar in the **Data Analysis** window. Adjust the report according to your needs.