

QuickGuide: RealFast™ Virus Detection on Bio-Rad CFX96

Setup for the SARS-CoV-2 RealFast™ Assay:

- Open the BioRad 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.

1	50.0	C for 10:00
2	95.0	C for 3:00
3	95.0	C for 0:15
4	57.0	C for 0:30
+ Plate Read		
5	GOTO 3	.44 more time
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, HEX and Cy5**.
 - 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 the SARS-CoV-2 Positive Control.
 - In the field **Target Names** check boxes to load fluorophores **FAM, HEX and Cy5**.
 - Type target names (FAM = SARS-CoV-2 specific [N gene], HEX= SARS-CoV-2 specific [RdRP/ORF1ab gene], Cy5 = human RNA Control [ACTB gene]) and sample name and press **Enter**.
 - in case of testing samples in replicates: click check box to load **Replicate** number
 - Press **OK** and save the plate file.
- Press **Next** and load your PCR tubes; start the run.

Analysis of the SARS-CoV-2 RealFast™ Assay:

- Open the data file: **File > Open > Data File**.
- Select the **Quantification** tab
 - Select the negative control template and - if needed - manually adjust the **threshold** (click & drag) above the background fluorescent signal.
 - Review your samples individually. Interpretation of the samples according to section **8. Data Analysis / Interpretation of Results** in the Instruction For Use.
 - FAM = SARS-CoV-2 specific amplification
 - HEX = SARS-CoV-2 specific amplification
 - Cy5 = human RNA specific amplification
- 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.

