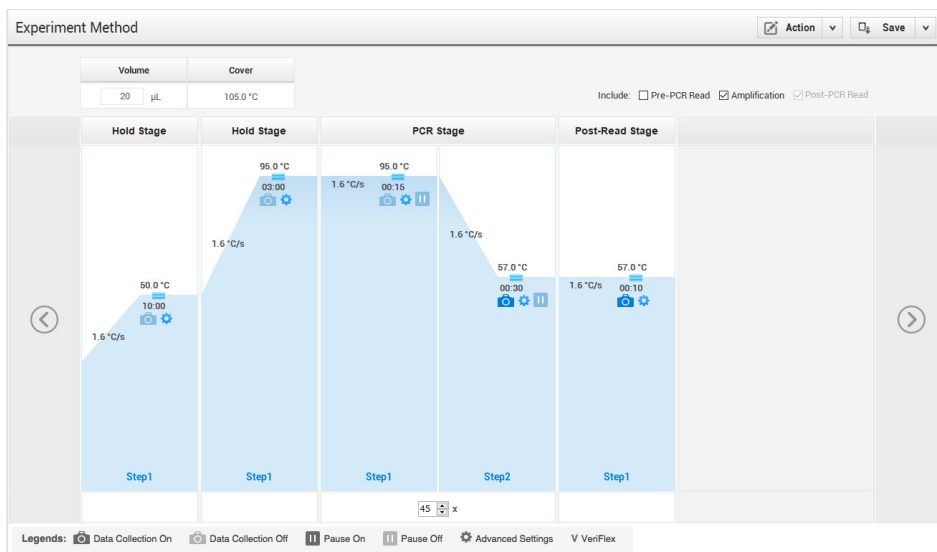


# QuickGuide: RealFast™ Virus Detection on AB QuantStudio 5

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

- Open the QuantStudio Design & Analysis Software (Quickguide is based on version 1.5.2) and click **File/New Experiment/Experiment Setup**.
- Define **Experiment Properties**
  - Name: Name of experiment
  - Instrument type: **QuantStudio 5 System**
  - Block type: according to your instrument
  - Experiment type: **Presence/Absence**
  - Chemistry: **TaqMan® Reagents**
  - Run mode: **Standard**
- In **Method** define the temperature / time profile of the RT-PCR, the volume of the reaction and the number of cycles according to the Instruction For Use of the SARS-CoV-2 RealFast™ Assay:



- In **Plate / Quick Setup** under **Passive Reference** select **None**.
- In **Plate / Advanced Setup** define the **Targets** (name of the targeted gene, reporter, quencher and color) and **Samples**. To add a new target or sample click **+ Add**.

Assign Targets and Samples

Quick Setup    **Advanced Setup**

Targets						
	Name	Reporter	Quencher	Co...	Task	Quantity
<input checked="" type="checkbox"/>	N Gene	FAM	NFQ-MGB		U	X
<input checked="" type="checkbox"/>	RdRP/ORF1ab	VIC	NFQ-MGB		U	X
<input checked="" type="checkbox"/>	ACTB	CY5	NFQ-MGB		U	X

Samples			
	Sample Name	Comments	
<input type="checkbox"/>	Sample 1		X
<input type="checkbox"/>	Sample 2		X
<input type="checkbox"/>	Sample 3		X

Assign the samples and targets to selected wells by click + drag in the **Plate Layout**. Tick the respective sample name as well as targets to be detected.

### Define your **No Template Control**:

- Select two NTC wells in the plate layout by click+drag.
- Select "N" for all targets in the **Task** field.



### Define your **Samples** and **Positive Control**:

- Select a well in the plate layout.
- Assign sample to selected well by ticking the check box.
- Select "U" for all targets in the **Task** field.

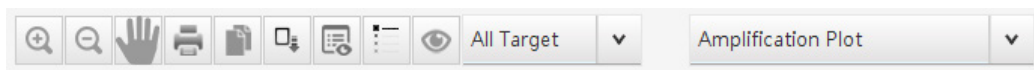



- In **Run** save the experiment, load your PCR tubes and press the **START RUN** button.

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

After completing a run or after **Open Existing Experiment** data file the software displays **Results**:

Menu bar:



- For display mode select **Amplification Plot** and click the **eye icon** to adjust the plot settings:
  - Plot type: **ΔRn Vs. Cycle**
  - Graph Type: **Linear**
  - Plot Color: **Target**
- Click on the **Settings** icon in the top-right corner next to the **Analyze** button:
  - Within the **CT Settings** tab disable the use of the **Default Settings** then the **Automatic Threshold** and adjust the threshold manually above the background signals of the **No Template Control** for each channel.
  - Disable **Automatic Baseline**. **Baseline Start Cycle** should be set to "3" and **Baseline End Cycle** to the cycle immediately preceding the rise of the first amplification curve.
  - Confirm with pressing **Apply**.
  - The threshold can only be displayed in the **Amplification Plot** for each target separately (selection of the target in the drop-down menu **All Target**).
- Click on the **Analyze** button.
- Review your samples by selecting individual wells in **Plate Layout** or by clicking the **Action** button and **Select sample**.
  - The display of the results can be viewed either in table or plate layout format by selecting: 
  - Adjust the displayed details according to your needs by selecting/deselecting the listed features in **View**.
- To print a report click **File** in the upper menu bar and go to **Print Report**:
  - Select data for the report according to your needs.
- In **Export** results can be exported to an Excel or text file:
  - Choose a File Type and adjust the Content according to your needs by ticking the respective boxes.

