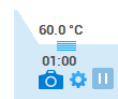


## QuickGuide: RealFast™ Variant Detection on AB QuantStudio 5

### Setup for Variant Detection and Onco RealFast™ Assays:

- Open the QuantStudio Design & Analysis Software and click **Create New Experiment**.
- In **Experiment Properties** define
  - Name: Name of experiment
  - Instrument type: **QuantStudio 5 System**
  - Block type: **96-Well 0,2 ml Block**
  - Experiment type: **Quantitation – Standard Curve**
  - Chemistry: **TaqMan® Reagents**
  - Run mode: **Standard**
- In **Method** select a sample volume of **20 µl** and setup the **PCR program** according to the Instructions for Use (IFU) of your RealFast™ Assay.
  - Make sure **Data Collection On** is enabled.



- In **Plate** → **Quick Setup** → **Plate Attributes** define **None** as **Passive Reference** dye for Variant Detection RealFast™ Assays (e.g. HLA-B27, HLA-B5701, other HLA-Assays.....) only.

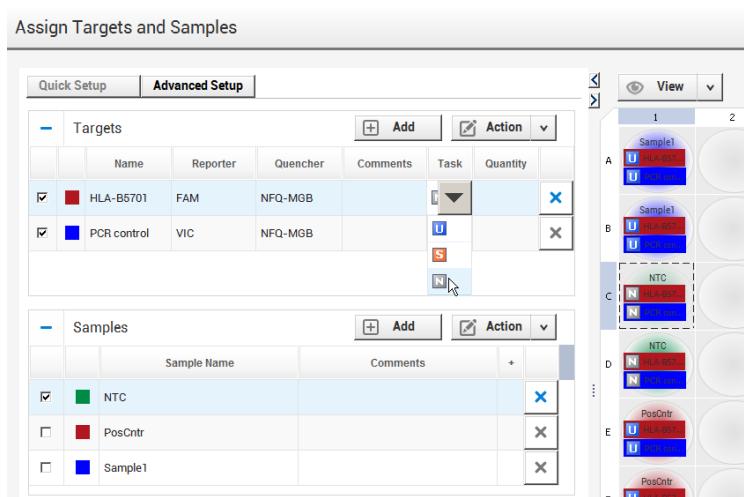
Select **ROX** as passive **reference dye** for Onco RealFast™ Assays (e.g. EGFR T790M,.....) only.



- In **Plate** → **Advanced Setup** assign **Targets** and **Samples** to selected wells.
  - Select **Targets** and edit and enter assays. Keep default settings for reporter dyes:
    - Target 1 = FAM (corresponds to FAM-labeled probe). Quencher = NFQ-MGB
    - Target 2 = VIC (corresponds to HEX-labeled probe). Quencher = NFQ-MGB

» **Note:** In case you want to run several **different** RealFast™ Assays in parallel, add targets for each of the markers to be analyzed. For assignment of fluorophores to hydrolysis probes see Instructions for Use of the respective RealFast™ Assay. «

- Click **Add** repeatedly to enter all your samples and controls. Assign **Targets** and **Samples** and select the total number of wells by click+drag in the plate layout.
- Define your **No Template Control (NTC)**. Select a replicate (2 wells) in the plate layout by click+drag. Select „**N**“ for both targets in the **Task** field.
- Define your **Samples** and **Controls**. Select well(s) in the plate layout. Assign sample(s) to selected well(s). Select „**U**“ for both targets in the **Task** field.



- **Save** the experiment.
- Load your PCR tubes / plate into the QuantStudio™ instrument and press **START RUN** button to start the run.

## Analysis of Variant Detection and Onco RealFast™ Assays:

After completing a run or after opening an **Existing Experiment** the software displays the **Results** tab.

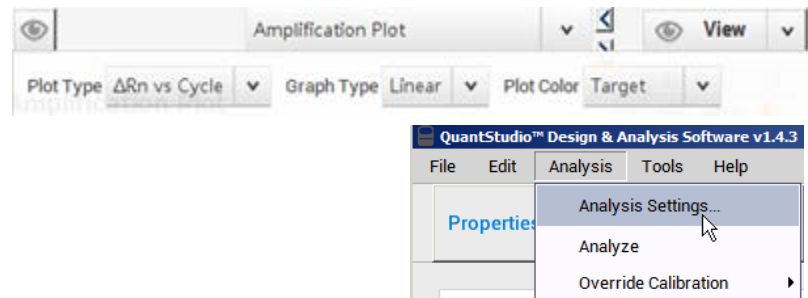
- The **Amplification Plot** appears automatically.
- If you have **different** RealFast™ Assays in one run, select the appropriate target by clicking **Action** and using the dropdown menu.
- Press the button **Show Plot Settings** that is indicated by an eye-symbol and adjust the parameters:

➤ Plot Type: **ΔRn Vs. Cycle**

➤ Graph Type: **Linear**

➤ Plot Color: **Target**

➤ Move to **Analysis Settings**.



For **Variant Detection** RealFast™ Assays (e.g. HLA-B27, HLA-B5701, other HLA-Assays,...) only:

Disable **Default Settings**, **Automatic Threshold** and **Automated Baseline**. Select the individual targets and adjust the **Threshold** according to the IFU. **Baseline Start Cycle** should be set to „3“ and **Baseline End Cycle** to „15“.

For **Onco RealFast™** Assays (e.g. EGFR T790M,...) only:

Enable **Automatic Threshold** and **Automated Baseline**.

» **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 IFU. «

- Confirm the analysis settings by pressing the **Apply** button.
- Review your samples by selecting individual wells in the plate layout.

» **Note:** *Threshold settings of individual targets can be alternatively adjusted in the **Show Plot Settings** window.* «

➤ To show results as table click the **Well Table** icon in the upper right corner. Adjust the table according to your needs by selecting/deselecting the listed features in the **View** dropdown menu.

➤ Verify absence of any contamination in the **Negative Control**. No amplification should be visible.

The screenshot shows the 'Well Table' view with a table of results. The table has columns for #, Well, Sample Na..., Target Na..., Task, and Ct. The 'View' dropdown is set to 'Table'.

#	Well	Sample Na...	Target Na...	Task	Ct
1	A1	NTC	HLAB27	NTC	Undeter...
1	A1	NTC	PCR Cont...	NTC	Undeter...
2	A2	pos. Contr...	HLAB27	UNKNOWN	20.815
2	A2	pos. Contr...	PCR Cont...	UNKNOWN	21.401

- To print a report select **File > Print Report** in the upper menu bar:
  - Select data for the report according to your needs.
  - If you wish to export your data go to the **Export** tab and choose the file type and content