



QuickGuide: RealFast™ Variant Detection on AB 7500 Fast

Setup for Variant Detection and Onco RealFast™ Assays:



- Open the **AB 7500 Software** (QuickGuide is based on version 2.0.6) and click **Advanced Setup**. In the Experiment Menu go to **Setup**.
- In **Experiment Properties** define Experiment Name: **Name**
Instrument: **7500 (Fast)**
Type of experiment: **Quantitation – Standard Curve**
Reagents: **TaqMan® Reagents**
Ramp speed: **Standard**
- In **Plate Setup** assign **Targets** and **Samples** to selected wells.
 - In **Define Targets** click **Add New Target**, name your targets and assign the correct **Reporter** and **Quencher** (i.e. FAM for targeted variant and VIC for PCR control):



Define Targets			
Target Name	Reporter	Quencher	Colour
HLA-B27	FAM	NFQ-MGB	
PCR Control	VIC	NFQ-MGB	

- Click **Add New Sample** repeatedly to enter all your samples and controls.
 - Click **Assign Targets and Samples** and select the total number of wells by click+drag in the **View Plate Layout**.
 - Assign targets to selected wells by ticking the **Assign** box in the **Assign target(s) to** field.
- » **Note:** Select **None** as passive **reference dye** for **Variant Detection RealFast™** Assays (e.g. HLA-B27, HLA-B5701) only.

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

- 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 a well in the plate layout.
 - Assign sample to selected well by ticking the **Assign** box in the **Assign sample to** field.
 - Select "U" for both targets in the **Task** field.
- In **Run Method** select a sample volume of **20 µl** and setup the PCR program according to the Instructions for Use of your RealFast™ Assay. Make sure **Data Collection On** is enabled.
- **Save** the experiment.
- Load your PCR tubes / plate and press **START RUN** (green button) to start the run.

Assign target(s) to the selected wells.		
Assign	Target	Task
<input checked="" type="checkbox"/>	HLA-B27	
<input checked="" type="checkbox"/>	PCR Control	

Assign target(s) to the selected wells.		
Assign	Target	Task
<input checked="" type="checkbox"/>	HLA-B27	
<input checked="" type="checkbox"/>	PCR Control	

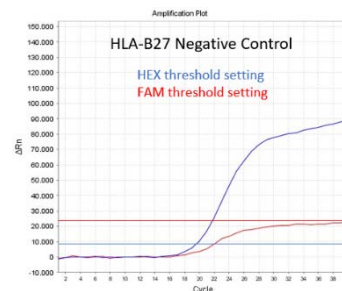
Analysis of Variant Detection and Onco RealFast™ Assays:

After completing a run or after opening a data file the software displays the Experiment Menu **Analysis**:

- Adjust the **Plot Settings** in the field called **Amplification Plot**:
 - Use plot type: **ΔRn Vs. Cycle**
 - Graph Type: **Linear**
 - Color: **Target**
- Click on the **Analysis Settings** window in the top-right corner:

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

- Within the **CT Settings** tab disable the **Automatic Threshold** and adjust the threshold manually above the background signals of the **Negative Control** (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).
- Disable **Automatic Baseline**. **Baseline Start Cycle** should be set to "3" and **Baseline End Cycle** to "15".
- Confirm with pressing **Apply Analysis Settings**.



Select a Target

Target	Threshold	Baseline Start	Baseline End
HLA-B27	25.000	3	15
PCR Control	10.000	3	15

CT Settings for the 2 Selected Targets

CT Settings to Use: Use Default Settings

Automatic Threshold

Threshold:

Automatic Baseline

Baseline Start Cycle: End Cycle:

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

- Within the **CT Settings** tab enable the **Automatic Threshold**.
- Enable **Automatic Baseline**.
- Confirm with pressing **Apply Analysis Settings**.

Select a Target

Target	Threshold	Baseline Start	Baseline End
EC	AUTO	AUTO	AUTO
EGFR c.2369C>T (p.T790M)	AUTO	AUTO	AUTO

CT Settings for the 2 Selected Targets

CT Settings to Use: Use Default Settings

Automatic Threshold

Threshold:

Automatic Baseline

Baseline Start Cycle: End Cycle:

» **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*. «

- Review your samples by selecting individual wells in **View Plate Layout**.
- To show results as table click **View Well Table**.
 - Adjust the table according to your needs by selecting/deselecting the listed features in **Show in Table**.
- To print a report click **Print Report** in the upper menu bar:
 - Select data for the report according to your needs.
- To export results in an Excel or text file click **Export...** in the upper menu bar:
 - Define **Export Properties** and **Customise Export**.

