



QuickGuide: RealFast™ Variant Detection on AB 7500 Fast

Setup for Variant Detection and Onco RealFast™ Assays:







- Open the AB 7500 Software 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			
<input type="button" value="Add New Target"/> <input type="button" value="Add Saved Target"/> <input type="button" value="Save Target"/> <input type="button" value="Delete Target"/>			
Target Name	Reporter	Quencher	Colour
HLA-B5701	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.

Assign	Target	Task
<input checked="" type="checkbox"/>	HLA-B5701	  
<input checked="" type="checkbox"/>	PCR control	  

- 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.

Assign	Target	Task
<input checked="" type="checkbox"/>	HLA-B5701	  
<input checked="" type="checkbox"/>	PCR control	  

- 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.
- **Save** the experiment.
- Load your PCR tubes / plate and press **START RUN** (green button) to start the run.

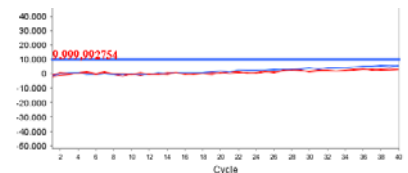
Analysis of Variant Detection and Onco RealFast™ Assays:

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**.
- Disable **Automatic Baseline**. **Baseline Start Cycle** should be set to „3“ and **Baseline End Cycle** to „15“.
- Confirm with pressing **Apply Analysis Settings**.



Target	Threshold	Baseline Start	Baseline End
HLA-B5701	10.000	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**. «

Target	Threshold	Baseline Start	Baseline End
Mut EGFR	AUTO	AUTO	AUTO
POLRIIA	AUTO	AUTO	AUTO

CT Settings for Mut EGFR

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**.

