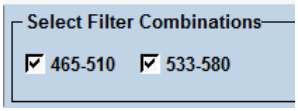
QuickGuide: RealFast™ Variant Detection on LightCycler® 480II

Setup for Variant Detection and Onco RealFast™ Assays:

- Open the **LightCycler® 480 software** (QuickGuide is based on version 1.5.1) and login with your username and password.
  - Choose **New Experiment**.
- Define your PCR program in the **Run Protocol** tab.
  - Select **Dual Color Hydrolysis Probe / UPL Probe** as Detection Format.
  - Select a **reaction volume** of 20 µl and setup the **Program**:
  - Add programs in the **Program Name** window with "**+**" and edit **Cycles** and **Analysis Mode**. To edit **Acquisition Mode** and **Hold**, click on the corresponding step in the **Program Name** window and change parameters or add steps ("**+**") in the **Temperature Targets** window.
  - Setup the PCR program according to the Instructions for Use of your RealFast™ Assay.
- Click on **Subset Editor** button on the left side of the window.
  - Press "**+**" to create a new subset and rename your subset.
  - Select wells in the grid and press **Apply**.
- Click on **Sample Editor** button on the left side of the window.
  - **Select Workflow > Abs Quant.**
  - **Select Filter Combinations > 456-510** nm (FAM) and **533-580** nm (HEX).
  - Choose your **Subset** of Samples.



- Define your **No Template Control (NTC)**:
  - **Select Samples** field: select well by mouse-click or two wells by ctrl + mouse click.
  - **Edit Abs Quant Properties** field: Type **NTC** in the **Sample Name** field and press **Enter**. Choose **Negative Control** as **Sample Type**.

	Pos	Filter combination	Color	Repl Of	Sample Name	Quantification Sample Type	Concentration
▸	A1	FAM (465-510		A1	NTC	Negative C	
	A1	VIC / HEX /		A1	NTC	Negative Con	
	A2	FAM (465-510		A1	NTC	Negative Con	
	A2	VIC / HEX /		A1	NTC	Negative Con	

- Define your **Samples:**
  - **Select Samples** field: select a well by mouse click.
  - **Edit Abs Quant Properties** field: Type the name of your first sample in the corresponding field and press **Enter**. Check **Unknown**.
  - Define the rest of your samples.

	Pos	Filter combination	Color	Repl Of	Sample Name	Quantification Sample Type	Concentration
▸	A3	FAM (465-510		A3	3	Unknown	
	A3	VIC / HEX /		A3	3	Unknown	
	A4	FAM (465-510		A3	3	Unknown	
	A4	VIC / HEX /		A3	3	Unknown	

- **Save** or **export** your experiment by pressing the corresponding button:

- Load your samples and start the experiment.

Analysis of Variant Detection and Onco RealFast™ Assays using "Abs Quant/Fit Points":

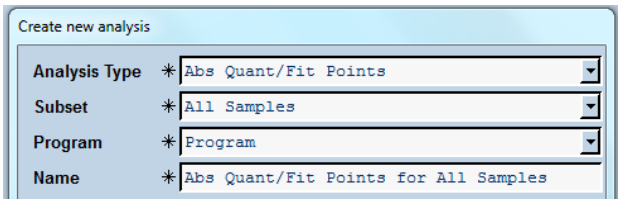
- Open the **LightCycler® 480 software** and login with your username and password. The **Overview** window appears.
- Click on  or choose **Navigator** in the flip-window on the top left.

  - Choose an experiment from the data bank or
  - import an experiment located outside the data bank by pressing **Import.**

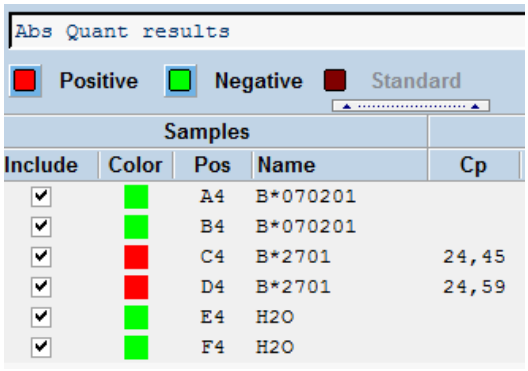
- After the file is loaded the **Summary** window of your experiment is displayed.

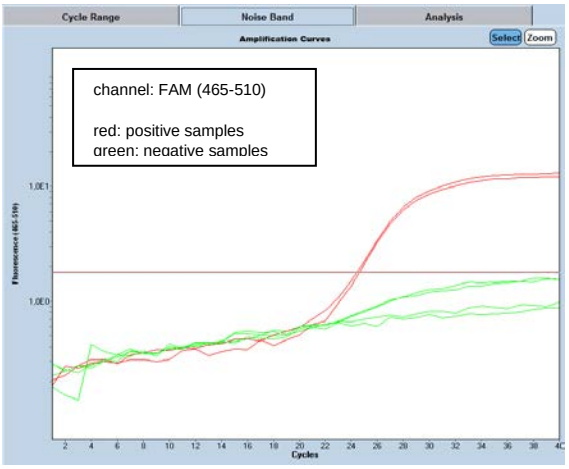
- Press the **Analysis** button to reach the analysis window.

  - Within the **Create New Analysis** field choose **Abs Quant/Fit Points.**
  - A pop-up window will be launched. If applicable select a **Subset** of samples or analyze **All Samples** in case your plate contains only one type of assay. Give a **Name** to your analysis. Press the **OK** button



  - *Optional:* **Press Color Compensation** and choose **In Use** for color compensation of FAM (510) and VIC (580). Within the pop-up window select a color compensation and press the **OK** button.
  - Press **Filter Comb 465-510**, choose **FAM (465-510)** in the pop-up window and press the **OK** button.
  - Within the **Noise Band** field adjust the threshold manually. Set the threshold in the exponential phase of the amplification curves above the background.





  - Press the **Calculate** button. Choose **Abs Quant Results** in the chart bottom-left and review your samples. Samples positive for the targeted variant show an amplification in the FAM channel and will be color-coded.
  - To review proper amplification of all samples, press **Filter Comb 465-510**, choose **VIC / HEX / Yellow 555 (533/580)** in the pop-up window and press the **OK** button.
  - Press the **Calculate** button and review your results in the chart bottom-left. All genomic DNA samples and Controls show an amplification in the HEX channel and are therefore marked as positive.

» ***Note****: Set threshold value for the FAM channel just above the background fluorescent signal generated by the e.g. HLA-B27 Negative Control. 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 respective Instructions for Use. «*

- **Save** or **export** your data by pressing the corresponding button.  or 

- After saving your data you can customize and generate a report via the **Report** button.

Optional:

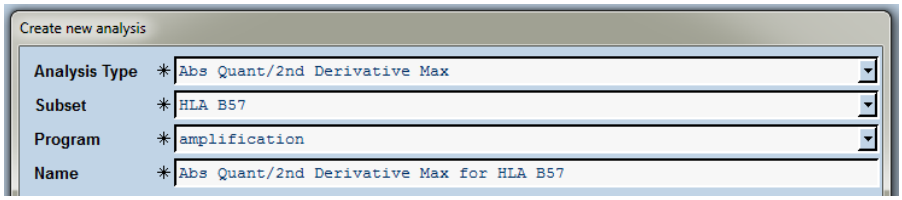
<u>Analysis of Variant Detection and Onco RealFast™ Assays using Abs Quant/2nd Derivative Max:</u>

- Open the **LightCycler® 480 software** and login with your username and password.
  The **Overview** window appears.
- Click on  or choose **Navigator** in the flip-window on the top left.
  - Choose an experiment from the data bank or
  - import an experiment located outside the data bank by pressing **Import.**

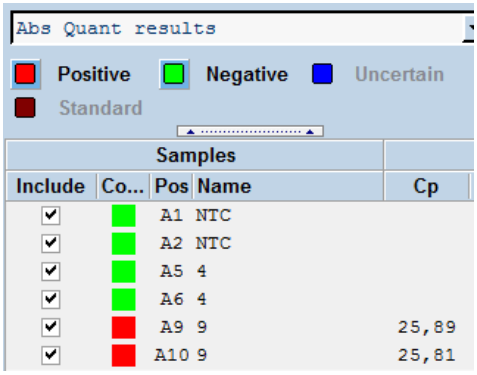
- After the file is loaded the **Summary** window of your experiment is displayed.

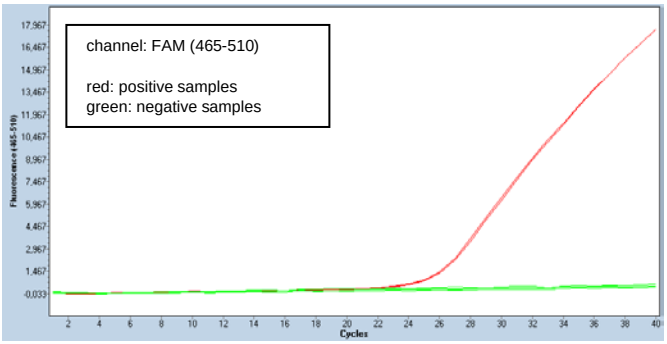
- Press the **Analysis** button to reach the analysis window.

  - Within the **Create New Analysis** field choose **Abs Quant/2nd Derivative Max**.
  - A pop-up window will be launched. If applicable select a **Subset** of samples or analyze **All Samples** in case your plate contains only one type of assay. Give a **Name** to your analysis. Press the **OK** button.



  - *Optional:* **Press Color Compensation** and choose **In Use** for color compensation of FAM (510) and VIC (580). Within the pop-up window select a color compensation and press the **OK** button.
  - Press **Filter Comb 465-510**, choose **FAM (465-510)** in the pop-up window and press the **OK** button.
  - Press the **Calculate** button. Choose **Abs Quant Results** in the chart bottom-left and review your samples. Samples positive for the targeted variant show an amplification in the FAM channel and will be color-coded.





  - To review proper amplification of all samples, press **Filter Comb 465-510**, choose **VIC / HEX / Yellow 555 (533/580)** in the pop-up window and press the **OK** button.
  - Press the **Calculate** button and review your results in the chart bottom-left. All DNA samples and the Positive Control show an amplification in the HEX channel and are therefore marked as positive.

*» **Note**: Set threshold value for the FAM channel just above the background fluorescent signal generated by the e.g. HLA-B27 Negative Control. 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 respective Instructions for Use. «*

- **Save** or **export** your data by pressing the corresponding button.  or 

- After saving your data you can customize and generate a report via the **Report** button.