qPCR Cycler Programs for Venor®GeM qOneStep Kit

The following protocols were created based on internal experience and customer reports. Minerva Biolabs does not warrant or assume responsibility for the performance of these protocols.

Programming the LightCycler® 2.0

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	95
Incubation time [min]	2:00
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target Temperature [°C]	95	55	60
Incubation time [s]	15	30	45
Temperature Transition Rate [°C/s]	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None		
Single	None		

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Result Reading:

- Select the fluorescence channels 1 and 2
- Click on *Quantification* to generate amplification plots and Ct-values
- The threshold will be generated automatically.
- Samples showing no significant increase in the amplification plot can be considered as negative.

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Programming of ABI Prism® 7500

Detector Settings:

Target Probe:	Reporter - FAM™ / Quencher - none
Internal Control Probe:	Reporter - HEX™ / Quencher - none

The “ROX Reference” function needs to be disabled, as no ROX™ dye is included in the mix. Activate both detectors for each well.
Measure fluorescence during annealing.

Program Step 1: Pre-incubation

Setting	Hold
Temperature	95 °C
Incubation time	3:00 min

Program Step 2: Amplification

Cycles	45
Setting	Cycle
Denaturing	95 °C for 30 sec
Annealing	55 °C for 30 sec & data reading
Extension	60 °C for 45 sec

Result Reading:

- Enter the following basic settings at the right task bar:
  - Data: Delta RN vs. Cycle
  - Detector: FAM™ and HEX™
  - Line Colour: Well Colour
- Open a new window with for the *Graph settings* by clicking the right mouse button
  - Select the following setting and confirm with ok:
  - Real Time Settings: Linear
  - Y-Axis Post Run Settings: Linear and Auto Scale
  - X-Axis Post Run Settings: Auto Scale
  - Display Options: 2
- Initiate the calculation of the Ct-values and the graph generation by clicking on Analyze within the report window.
- Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- Samples showing no Ct-value can be considered as negative.

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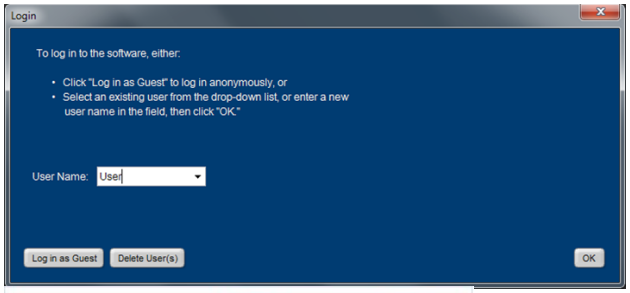
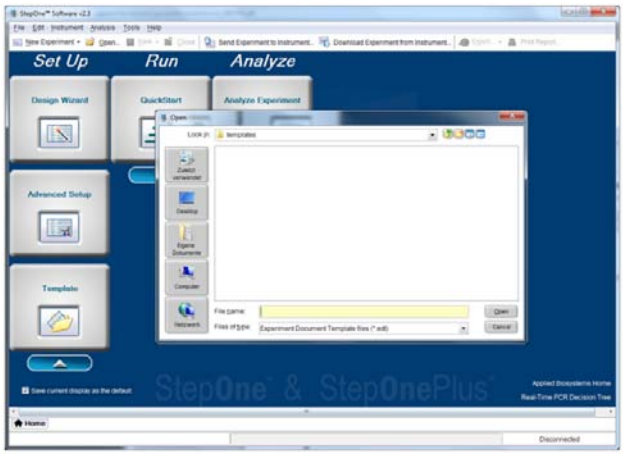
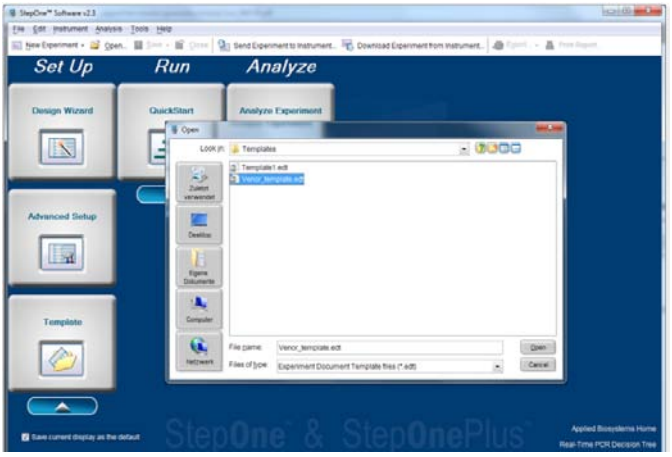
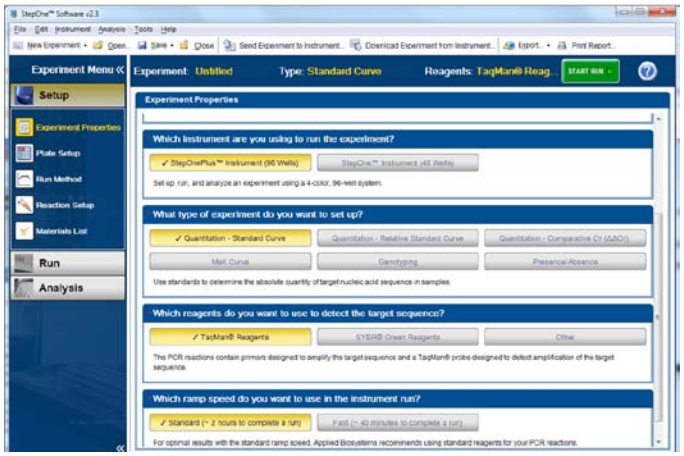
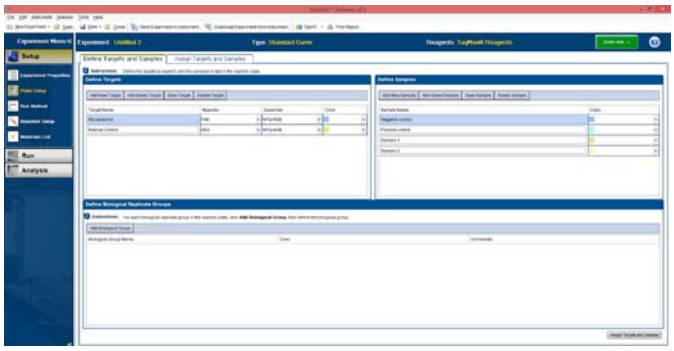
Programming the ABI StepOne™ / StepOne Plus™

Instrument setup using a template file:

1	If your device is using the StepOne™ Software v2.3, download the template file “Venor_template_StepOnePlus.edt” and/or “Venor_template_StepOne.edt” from our web page (www.minerva-biolabs.com/en/detection/venor-gem-qep) and save it to a folder on your hard drive or your network.	
2	Start the StepOne™ Software	 
3	For setting up a new experiment using setup information from a template click on the Template button. A new window will pop up.	

4	Navigate to the folder containing the template and open the appropriate template file (Venor_template_StepOnePlus.edt for StepOnePlus 96 well instrument and Venor_template_StepOne.edt for StepOne 48 well instrument, respectively.)	
5	Presented parameters in the sections Experiment Properties, Plate Setup and Run Method will be loaded.	
6	In the Plate Setup section further sample names can be defined. (Negative control and Positive control are already preset.)	

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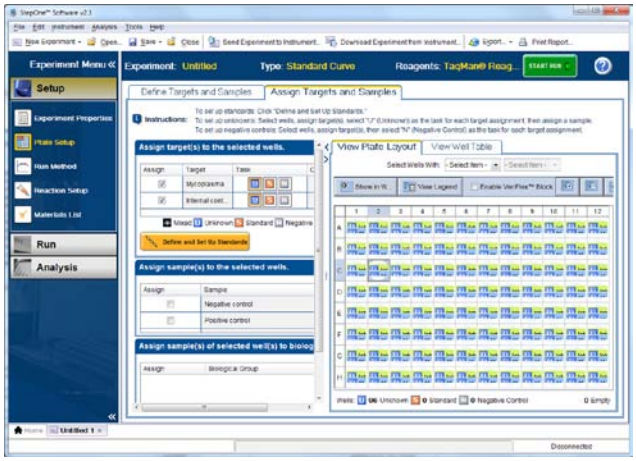
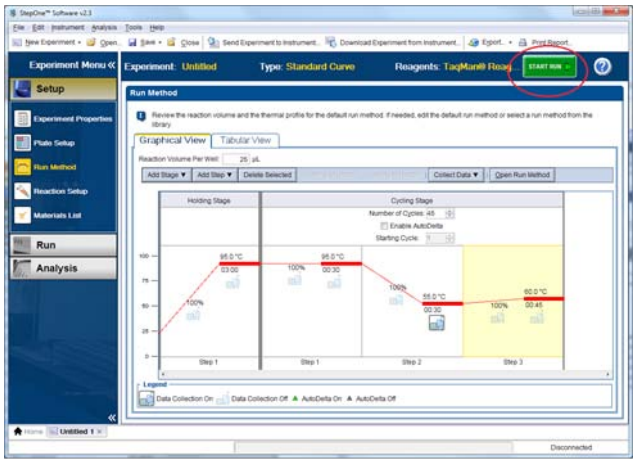
7	For assigning the names and/or another task (negative control, unknown, standard) to the wells switch to the tab „Assign Targets and Samples“.	
8	The cycling program is uploaded automatically. Click on start for PCR after loading the samples in the tray.	

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qPCR Cycler Programs for Venor®GeM qOneStep Kit

Programming of Rotor-Gene® 6000 (5-plex)

Program Step 1: Pre-incubation

Setting	Hold	**Please check the correct settings for the filter combination:**
Hold Temperature	95 °C	**green filter (510): Mollicutes**
Hold Time	3 min 0 sec	**yellow filter (555): Internal Control**

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95°C for 5 sec
Annealing	55°C for 30 sec → **acquiring to Cycling A (green and yellow)**
Elongation	60°C for 45 sec
Gain setting	automatic (auto Gain)
Slope Correct	activated
Ignore First	deactivated

Result Reading:

- Open the menu *Analysis*
- Select *Quantitation*
- Check the required filter set (green and yellow) according to the following table and start data analysis by double click.
- The following windows will appear:
  Quantitation Analysis - Cycling A (green or yellow)
  Quant. Results - Cycling A (green or yellow)
  Standard Curve - Cycling A (green or yellow)
- In window *Quantitation Analysis,* select first *linear scale* and than *slope correct*
  Threshold setup (not applicable if a standard curve was included in parallel and auto threshold was selected)
  - In window CT Calculation set the threshold value to 0-1
  - Pull the threshold line into the graph. Adapt the threshold line to the initial linear section of the positive control reaction.
- The Ct-values can be taken from the window *Quant. Results.*

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Programming the Mx3005P®

- Go to the setup menu, click on “Plate Setup”, check all positions which apply
- Click on “Collect Fluorescence Data” and check FAM™ and HEX™
- Corresponding to the basic settings the “Reference Dye” function should be deactivated
- Specify the type of sample (negative or positive control, sample, standard) at “well type”
- Edit the temperature profile at “Thermal Profile Design”:
  Segment 1: 95 °C for 3 min
  Segment 2:
  Denaturing 95 °C for 30 sec
  Annealing 55 °C for 30 sec & data collection end
  Extension 60 °C for 45 sec
  45 cycles
- at menu “Run Status” select “Run” and start the cycler by pushing “Start”

Analysis of raw data:

- In the window “Analysis” tab on “Analysis Selection / Setup” to analyze the marked positions
- Ensure that in window „algorithm enhancement“ all options are activated:
  Amplification-based threshold
  Adaptive baseline
  Moving average
- Click on “Results” and “Amplification Plots” for an automatic threshold
- Read the C_t values at “Text Report”

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Programming the LightCycler® 480

Program 1: Pre-incubation

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	95
Incubation time [min]	3:00
Temperature Transition Rate [°C/s]	4.4
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification

Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	***Segment 1***	***Segment 2***	***Segment 3***
Target Temperature [°C]	95	55	60
Incubation time [s]	30	30	45
Temperature Transition Rate [°C/s]	4.4	2.2	4.4
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	Single	None

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets	***Segment 1***
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	2.2
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Before starting the LC480, make sure that the filter setting is correct:

LightCycler® 480	Mollicutes	Internal Control
Instrument I	533 nm	568 nm
Instrument II	510 nm	580 nm

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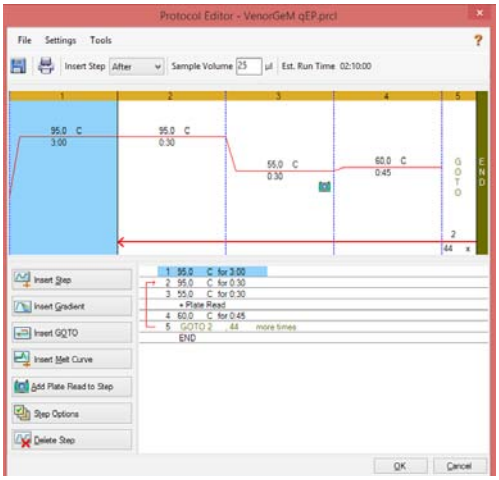
Programming the Biorad CFX 96™

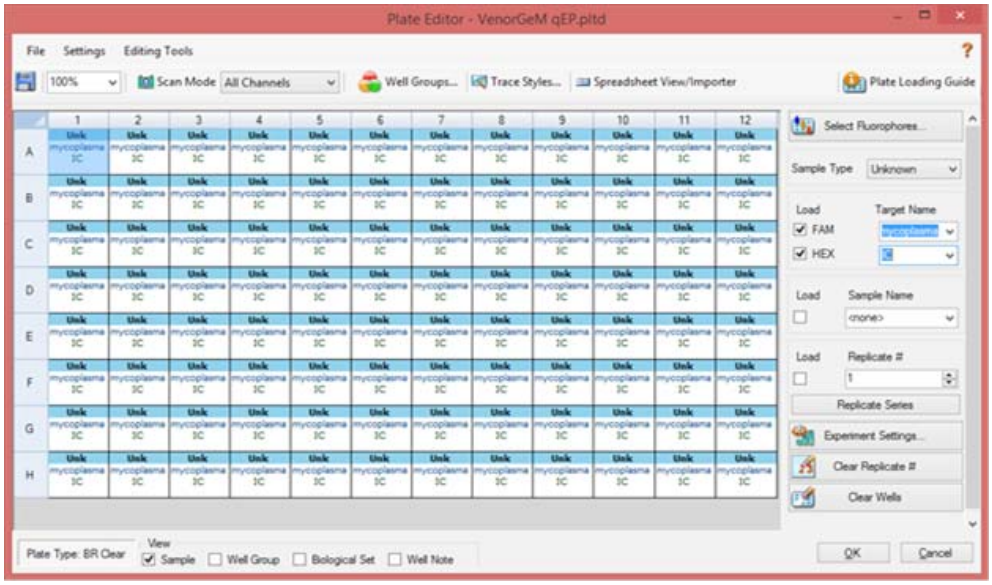
Run Setup Protocol Tab

Click **Create New** to open the *Protocol Editor* to create a new protocol.
Select any step in either the graphical or text display — the step becomes highlighted in blue. Click the temperature or well time to directly edit the value.



Run Setup Plate Tab

Click **Create New** to open the Plate Editor to create a new plate.



Use the **Scan Mode** dropdown menu in the Plate Editor toolbar to designate the data acquisition mode to be used during the run.

Important!!! Select the *All Channels* mode.

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Click **Select Fluorophores** to indicate the fluorophores that will be used in the run. Choose FAM™ for the detection of mycoplasma amplification and HEX™ for monitoring the amplification of the internal control. Within the plate diagram, select the wells to load.

Quantification Tab

The amplification chart displays traces of the relative fluorescence collected from each well at every cycle of the run.

Choose the fluorophore data you want to display by clicking the fluorophore checkboxes located under the amplification chart. Select FAM™ to display data of mycoplasma detection and select HEX™ to display internal control amplification data.

Data Analysis

The software uses two modes for quantification cycle determination. Select Settings from the menu bar and select *Baseline Subtracted Curve Fit* as baseline setting and *Single Threshold* mode as Cq Determination Mode. In the Single Threshold mode, click and drag the threshold line to manually position the line. Adapt the threshold line to the initial linear section of the positive control reaction.

Samples showing no C_t-value can be considered as negative.

Trademarks

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