Venor®GeM qEP

Mycoplasma Detection Kit for qPCR

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The Venor®GeM qEP kit is designed for the quantitative detection of Mollicutes, such as *Mycoplasma*, *Acholeplasma*, and *Spiroplasma,* in cell cultures and other biological matrices.

EXPLANATION OF THE TEST

The Venor®GeM qEP kit utilizes qPCR as the established method of choice for the rapid, robust and sensitive detection of Mollicutes. Using the qEP assay, you are able to detect all EP 2.6.7 required species in a single experiment. The assay is successfully tested with most qPCR devices. The assays' specificity is superior by targeting a highly conserved diagnostic target within the mycoplasma genomes.

The kit's design meets the test criteria of the *European Pharmacopoeia* (EP) with different kinds of sample material (chondrocytes, serum, cell culture supernatant, etc.). The assay is suitable for the direct detection in cell culture supernatants usually applicable in research, or for performing the "cell culture enrichment" method, or after DNA extraction. The kit fully complies with the requirements of EP 2.6.7.

TEST PRINCIPLE

Mollicutes species (see list in chapter "Assay Characteristics") are detected by amplifying the 16S rRNA coding region, whereas eukaryotic or other bacterial DNA is not amplified by the Venor®GeM qEP assay.

The user instructions include protocols for both screening of cell culture supernatant as well as EP compliant testing with defined DNA extraction and sample volume specifications. The entire test needs less than 3 hours, and, in contrast to methods like luminescence-linked enzymology, fluorescent staining or culture methods, there is no need for vital cells. Notably, the detection by PCR is considered to be superior in terms of sensitivity and precision.

False-negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA. The Internal Control DNA is either added directly to the PCR master mix to function as a PCR amplification control, or alternatively, added to the original sample prior to DNA extraction. By adding the Internal Control DNA directly to the sample, the DNA extraction and qPCR amplification is validated. Additional Internal Control DNA can be purchased from Minerva Biolabs (Order No. 11-9905). The internal control amplification is detected at 560 nm (HEX channel), whereas the mycoplasma-specific amplification is detected at 520 nm (FAM channel).

The kit contains dUTP instead of dTTP to facilitate precursor amplicon degradation by use of uracil-DNA glycosylase (UNG). Thus, the probability of false-positive result is minimized. Please note that UNG is not included in the Venor®GeM qEP kit.

REAGENTS

Each kit contains reagents for 25, 100, or 250 reactions. The expiry date of the unopened package is marked on the package label. The kit components must be stored at +2 to +8 °C until use. The rehydrated components must be stored at < -18 °C.

Component	Quantity			Cap colour
	25 reactions Order No. 11-9025	100 reactions Order No. 11-9100	250 reactions Order No. 11-9250	
Mycoplasma Mix	1 vial freeze-dried	4 vials freeze-dried	10 vials freeze-dried	red
Rehydration Buffer	1 vial 1.8 ml	1 vial 1.8 ml	3 vial 1.8 ml each	blue
Positive Control DNA	1 vial freeze-dried	1 vial freeze-dried	2 vials freeze-dried	green
Internal Control DNA	1 vial freeze-dried	2 vials freeze-dried	4 vials freeze-dried	yellow
PCR-grade Water	1 vial 2 ml	1 vial 2 ml	1 vial 2 ml	white

The lot specific Certificate of Analysis (CoA) can be downloaded from our website (www.minerva-biolabs.com).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Venor®GeM qEP kit contains reagents for the specific detection of Mollicutes. Additional consumables and equipment is supplied by the user:

- qPCR device with filter sets for detecting the fluorescence dyes FAM™ and HEX™
- PCR reaction tubes and caps for the specific qPCR device
- 1.5 ml reaction tubes, DNA- and RNA-free
- Microcentrifuge for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Required for EP 2.6.7 compliant testing:
  DNA extraction kit, e.g. Venor®GeM Sample Preparation Kit (e.g. order No. 56-1050)
  10 mM Tris-HCl buffer, pH 8.4
- Optional for process validation and EP 2.6.7 compliant testing:
  Internal Control DNA extra (order No. 11-9905)
  10CFU™ Sensitivity Standards available for all EP listed mycoplasma species (see last page)
  Proteinase K (order No. 56-0002)
- Optional for carry-over prevention:
  Uracil DNA glycosylase (UNG)

SAMPLES FOR CELL CULTURE SCREENING

Samples should be obtained from cell cultures that are highly confluent (90 % or higher). Cell culture supernatant is very well suited for the mycoplasma test without the need of additional sample preparation. However, PCR inhibiting substances may accumulate in the medium of cell cultures, which will make is necessary to extract the DNA prior to the PCR test (see below for further information). Note that penicillin or streptomycin in the culture media are not known to inhibit mycoplasma nor affect the tests' sensitivity.

The average mycoplasma number in cell culture is ~1 x 10^6 particles per ml with a maximum of 1 x 10^8 particles per ml. Within this range, a sufficient amount of mycoplasma DNA is present in the supernatant for successfully applying the PCR test. Prepare the PCR template as follows:

1.	Transfer 500 µl of the supernatant from the cell culture to a 1.5 ml reaction tube. Close the lid tightly.
2.	Incubate the sample at 95 °C for 10 minutes.
3.	Centrifuge the sample at max. speed briefly (15 s) to pellet cellular debris.
4.	Use 2 µl of the supernatant directly for qPCR, or store the sample for up to 6 days at 4 °C or at –20 °C for long time storage.

Cell pellets cannot be used directly for the test due to cell debris that will interfere with the PCR reaction. Thus cell pellets as well as foetal calf serum, vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction in advance. The Venor®GeM qEP assay was tested with these DNA extraction kits:

- Venor®GeM Sample Preparation kits (order No. 56-1010/-1050/-1200) for manual DNA extraction, or
- Venor®GeM Sample Preparation Kit - IP C16 (order No. 56-2096) for automated DNA extraction.

Extracted DNA may be stored at 4 °C for up to 6 days. Long time storage must be at ≤ –18 °C.

SAMPLES FOR EP 2.6.7 COMPLIANT TESTING

1. Sample concentration

For sample volumes from 200 to 1000 µl, a concentration step is recommended to achieve optimal sensitivity. Please note that the sample concentration works only with intact cells. Therefore, any step to disrupt cells such as heat inactivation prior to sample concentration must be avoided. Samples up to 200 µl volume can be processed directly without a concentration step.

1. Transfer up to 1 ml supernatant of the sample into a 1.5 ml reaction tube.
2. Centrifuge the sample at ≥ 10,000 x g for 15 min (or ≥ 13,000 x g for 6 min) to pellet mycoplasma particles.
3. Discard the supernatant and re-suspend the pellet in 200 µl Tris buffer (10 mM Tris-HCl, pH 8.4).
4. Vortex the sample briefly and proceed immediately with sample stabilization or DNA extraction.

2. Sample stabilization

Cell culture samples are likely to contain high concentrations of DNases which will degrade mycoplasma DNA even at lower temperatures. Therefore we recommend the following steps to stabilize the sample. This step is not necessary if DNA extraction is performed immediately after sample collection.

1. Transfer 500 µl of the supernatant from the cell culture into a 1.5 ml reaction tube. Close the lid tightly.
2. Incubate the sample at 95 °C for 10 minutes.
3. Centrifuge the sample at max. speed briefly (15 s) to pellet cellular debris.
4. Use the sample for DNA extraction. Store the sample for up to 6 days at 4 °C or at < –18 °C for long time storage.

3. DNA Extraction

A substantial body of evidence shows that DNA extraction is required to achieve the highest level of sensitivity. Numerous DNA extraction methods are established for a vast variety of sample materials. However, the DNA extraction method must be compatible for mycoplasma genomes. For EP compliant testing, the DNA method must be tested in combination with the PCR kit.

We recommend our Venor®GeM Sample Preparation kits (order Nos. 56-1010/-1050/-1200) for manual DNA extraction or the Venor®GeM Sample Preparation Kit - IP C16 (order No. 56-2092) for automated DNA extraction. These DNA extraction kits are tested intensively. The protocols for DNA extraction are described in detail in the instructions for users of the DNA extraction kits.

RECOMMENDATION

Ideally, the Internal Control DNA of the Venor®GeM qEP kit is used to verify the DNA extraction step as well.

Please note, that the actual sample volume that will be spiked is not relevant for the required volume of Internal Control DNA. The volume of Internal Control DNA depends on the final elution volume (containing the DNA extract) of the DNA extraction step. In general, add 2 µl per 10 µl DNA extract to the sample, vortex briefly and proceed with the DNA extraction. (Example: add 12 µl Internal Control DNA to the original sample if the elution volume will be 60 µl Elution Buffer.) Do not add further Internal Control DNA to the qPCR master mix if the internal control was already added to the sample before.

Note that this is not compatible with the sample concentration step as the sample concentration requires intact cells. Thus add the Internal Control DNA either prior to the sample stabilization or DNA extraction step.

Internal Control DNA can be purchased separately (Internal Control DNA extra, order No. 11-9905).

PRECAUTIONS

The Venor®GeM qEP kit is for in vitro use only. The kit should be used by trained laboratory staff only. All samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

IMPORTANT NOTES

⇒ These instructions must be understood to successfully use the Venor®GeM qEP kit. The reagents supplied should not be mixed with reagents from different batches and used as an integral unit. The reagents of the kit must not be used beyond their shelf life.

⇒ Follow the exact protocol. Any deviation may affect the test method and can affect the results.

⇒ PCR inhibition is likely to be caused by the sample matrix, or, in case of extracted DNA, caused by the elution buffer. Thus we recommend our Venor®GeM Sample Preparation kit for sample preparation. Any other DNA extraction kit needs to be qualified.

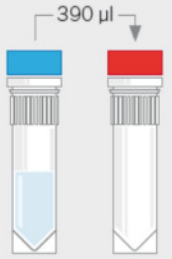
⇒ It is important to include control samples on a regular basis to monitor the reliability of your results. Positive and negative controls are essential in case of troubleshooting.

⇒ The control samples must be processed in the same manner as the test samples. You may want to include other laboratory specific control samples such as high, median and low DNA level (e.g. 3x LOD_{95}). Please note that Minerva Biolabs also offers to participate in external quality control programs.

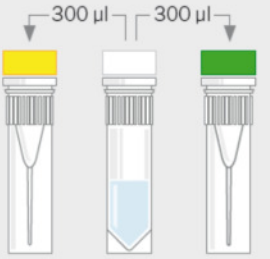
PROCEDURE - OVERVIEW

1. Spin Reagent Mix, Internal Control and Positive Control

2. Rehydration



a) Reagent Mix



b) Internal Control

c) Positive Control

3. Incubate 5 min at RT

4. Preparation of the reaction mix

EP compliant testing

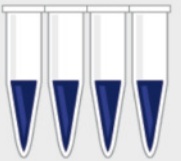
		1 reaction
Reagent Mix	red cap	15 µl
Internal Control	yellow cap	1 µl

Cell culture supernatant for screening

		1 reaction
Reagent Mix	red cap	15 µl
PCR grade water	white cap	8 µl
Internal Control	yellow cap	1 µl

5. Homogenize the reaction mix by carefully snipping

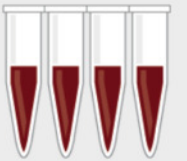
Loading the test tubes



15 µl reaction mix

+ 10 µl of extract **or** + 10 µl PC **or**
+ 10 µl Elution Buffer (NTC)

Loading the test tubes



23 µl reaction mix

+ 2 µl cell culture supernatant **or** + 2 µl PC **or**
+ 2 µl PCR grade water (NTC)

6. Place samples in a thermocycler and start PCR

Legend of cap colors

Reagent Mix
Rehydration Buffer
Internal Control
PCR grade water
Positive Control

PROCEDURE - STEP BY STEP

1. Reagent preparation

The test should be carried out with negative and positive controls and samples in duplicate. All reagents and samples must be equilibrated to +2 to +8 °C prior use. After reconstitution, the reagents should be stored at < -18 °C. Repeated freezing and thawing should be avoided and reconstituted controls (internal control and positive control) stored in aliquots.

1.	Mycoplasma Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Spin down all freeze-dried components at max speed for 5 sec.
2.	Mycoplasma Mix	Red cap	Add 390 µl Rehydration Buffer (blue cap) (For sample kits (5rxn): add 80 µl)
3.	Internal Control DNA	Yellow cap	Add 300 µl PCR-grade Water (white cap)
4.	Positive Control DNA	Green cap	Add 300 µl PCR-grade Water (white cap)
5.	Mycoplasma Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Incubate at room temperature for 5 min
6.	Mycoplasma Mix Internal Control DNA Positive Control DNA	Red cap Yellow cap Green cap	Vortex DNA briefly and spin for 5 sec

2. Reaction mix preparation

The following steps 2. to 4. (reaction mix preparation, add samples and start PCR amplification) should be done in less than 45 minutes to avoid a reduction in the fluorescent signal. Follow these schemes and sequences to set up the test:

1. Prepare the required volume of master mix for the number of samples. Mix the kit components at room temperature in a 1.5 ml reaction tube for all control and test reactions.

EP-compliant testing:

	Cap Colour	for 1 reaction	for 25 reactions
Mycoplasma Mix	Red	15 µl	375 µl
Internal Control DNA	Yellow	1 µl	25 µl

Cell culture screening:

	Cap Colour	for 1 reaction	for 25 reactions
Mycoplasma Mix	Red	15 µl	375 µl
PCR-grade Water	White	8 µl	200 µl
Internal Control DNA	Yellow	1 µl	25 µl

2. Homogenize the master mix by pipetting (5-times).

3. Pipet either 15 µl (for EP-compliant testing) or 23 µl (for cell culture screening) of the master mix to each PCR tube, discard remaining material.

3. Add samples

⇒ Set up positive and negative control samples (non template control) in duplicate in each PCR.

1.	Prepare non template controls: for EP-compliant testing: add 10 µl elution buffer supplied with the DNA extraction kit for cell culture screening: add 2 µl water or fresh cell culture medium or elution buffer
2.	Add test samples: for EP-compliant testing: add 10 µl of DNA extract for cell culture screening: add 2 µl of sample
3.	Prepare Positive Control: for EP-compliant testing: add 10 µl of rehydrated Positive Control DNA for cell culture screening: add 2 µl of rehydrated Positive Control DNA
4.	Spin PCR tubes briefly and ensure that all tubes are closed tightly.

4. Start PCR amplification

1.	Place PCR tubes in the qPCR device and close the lid.
2.	Program the qPCR cycler (see appendix I for detailed cycler programs of selected qPCR cyclers. Programs for additional cyclers are be available upon request.
3.	Start the program.

This assay was tested on the following qPCR devices:

qPCR device	**Manufacturer**
CFX-96	Bio-Rad
LightCycler® 2.0	Roche Diagnostics
ABI Prism® 7500	Applied Biosystems
RotorGene® 6000	Corbett Research
Mx3005P®	Agilent Technologies
AriaMx	Agilent Technologies

DATA INTERPRETATION

The presence of Mollicutes is indicated by an increasing fluorescence signal in the FAM channel. The quantification is based on threshold cycle (Ct) values and a DNA standard curve. The exact procedure for obtaining Ct -values including baseline calculation/normalization depends on the particular qPCR device and cycler control software. Please see the documentation of your device for further details. We recommend to assess the amplification curve progression of any sample including control samples.

A positive PCR is indicated by Ct $<$ 40. PCR reactions with Ct $\geq$ 40 are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM™ and/or the HEX™ channel (given the Internal Control was added). The Mollicutes DNA and Internal Control function as competitors in the PCR. Thus, the more Mollicutes DNA is in the sample, the higher the signal is in the FAM™ channel and the lower the internal control signal is in the HEX™ channel. The following matrix will help to interpret the PCR result:

Detection of Mollicutes FAM™ channel	Internal control HEX™ channel	Interpretation
positive	irrelevant	Mollicutes positive
negative	negative	PCR inhibition
negative	positive	Mollicutes negative

ASSAY CHARACTERISTICS

1. Sensitivity

The detection limit was determined by using the mycoplasma species listed in the EP 2.6.7 as template directly from culture broth (in parallel titrated by the traditional culture method).

Species	Detection limit LOD_{95} [CFU/ml]	Species	Detection limit LOD_{95} [CFU/ml]
Acholeplasma laidlawii	≤ 10 CFU/ml	*Mycoplasma pneumoniae*	≤ 10 CFU/ml
Mycoplasma hyorhinis	≤ 10 CFU/ml	*Mycoplasma arginini*	≤ 10 CFU/ml
Mycoplasma fermentans	≤ 10 CFU/ml	*Mycoplasma gallisepticum*	≤ 10 CFU/ml
Mycoplasma orale	≤ 10 CFU/ml	*Spiroplasma citri*	≤ 10 CFU/ml
Mycoplasma synoviae	≤ 10 CFU/ml		

2. Specificity

The qPCR assay will detect further Mollicutes species (see the following list), but not any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus* and *Streptococcus*. The waterborne germ *Burgholderia* is not detected. Cross-reactivity with other bacterial and mammalian DNA was tested negative:

Positively tested Mollicutes	Negatively tested		
	EP listed bacteria	Other bacteria	Mammals
Mycoplasma arthritidis	*Clostridium acetobutylicum*	*Chlamydia trachomatis*	Vero-B4
Mycoplasma genitalium	*Lactobacillus acidophilus*	*Legionella pneumophila*	Per.C6
Mycoplasma hominis	*Streptococcus pneumoniae*	*Micrococcus luteus*	RK13
Mycoplasma penetrans		*Candida albicans*	CHO-K1
Mycoplasma salivarium		*Enterococcus faecalis*	Murine genomic DNA
Ureaplasma urealyticum		*Enterobacter aerogenes*	Calf thymus DNA
		Escherichia coli	Foetal bovine serum
		Proteus mirabilis	Horse serum
		Burgholderia cepacia	Goat serum

APPENDIX I

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.

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

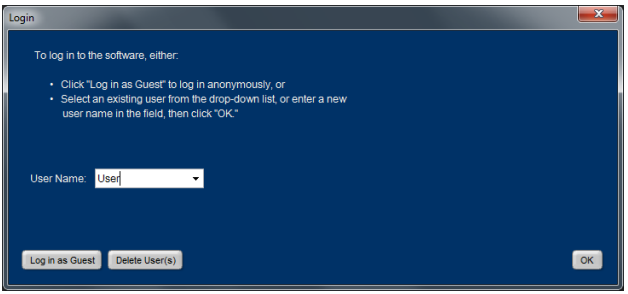
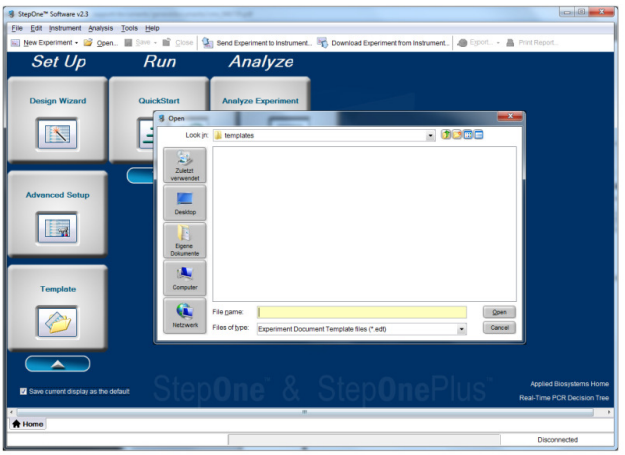
Program Step 2: Amplification

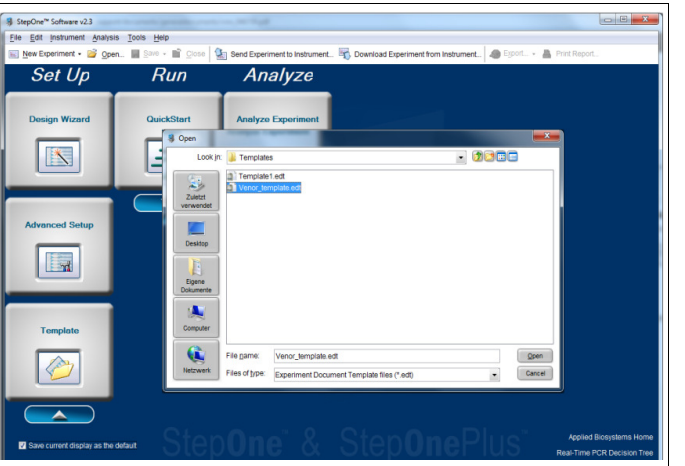
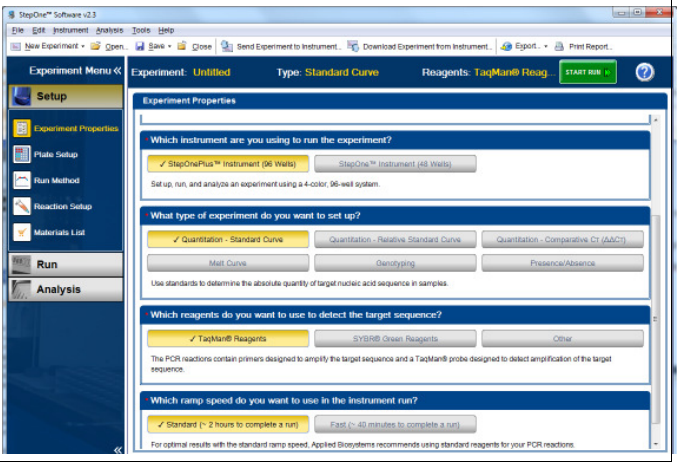
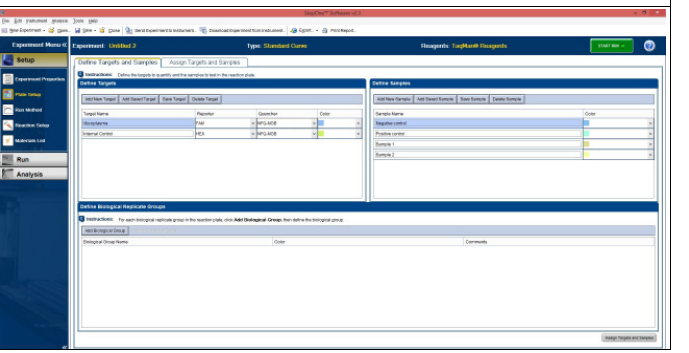
Cycles	45
Setting	Cycle
Denaturing	95 °C for 30 sec
Annealing	55 °C for 30 sec & data reading
Extension	60 °C for 45 sec

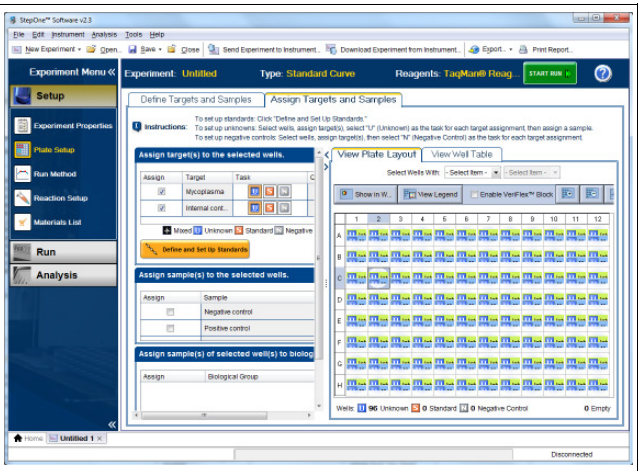
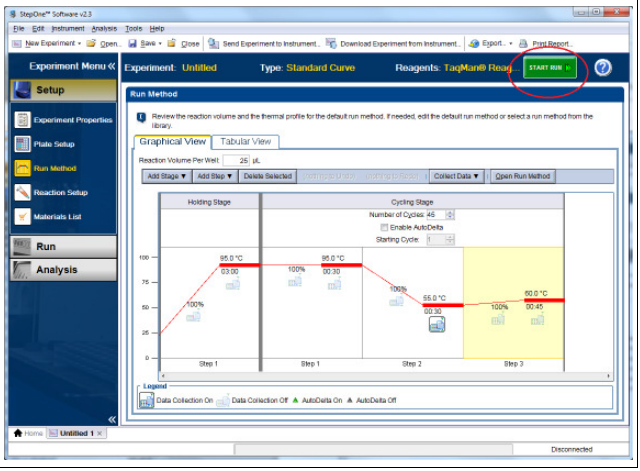
Result Reading:

- 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.

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_StepOne Plus.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.)	

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.	

Programming of RotorGene® 6000 (5-plex)

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95 °C
Hold Time	3 min 0 sec

Please check the correct settings for the filter combination:

green filter (510):	**Mollicutes**
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*.

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: 3 min, 95 °C
  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 Ct values at „Text Report“

Programming the LC 480

This protocol was created on the basis of internal experience and reports from customers. Minerva Biolabs does not warrant or assume responsibility for the performance of this protocol.

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:

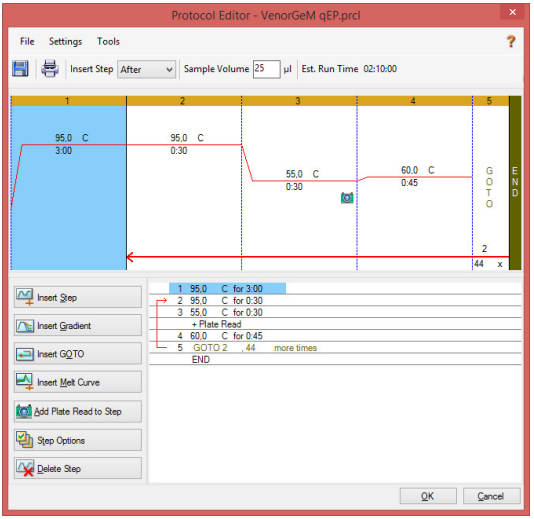
LightCycler 480	Mollicutes	Internal Control
Instrument I	533 nm	568 nm
Instrument II	510 nm	580 nm

Programming the Biorad CFX 96

Run Setup Protocol Tab

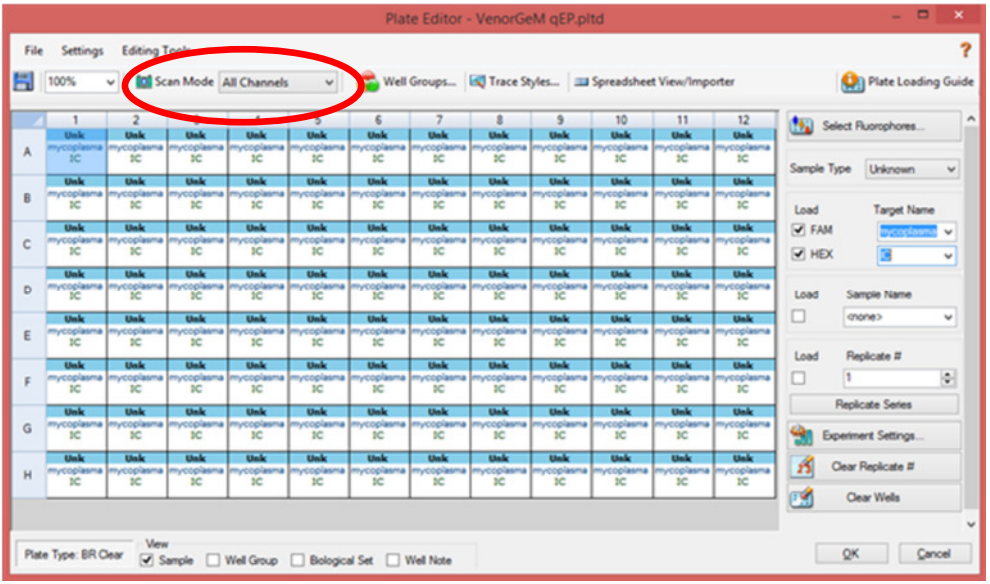
Click **Create New** to open the *Protocol Editor* to create a new protocol.

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.

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 Ct-value can be considered as negative.

APPENDIX II

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

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Related Products

MB *Taq* DNA Polymerase

53-0050/-0100/-0200/-0250	MB *Taq* DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB *Taq* DNA Polymerase (1 U/µl)	50/100/200/250 units

Contamination Control Kits for conventional PCR

11-1025/-1050/-1100/-1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 tests

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 extractions

Mycoplasma Elimination

10-0200/0500/1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 1 x 10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumonia*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See Minerva homepage for further available species

10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control

102-1003	*Mycoplasma arginini*	
102-2003	*Mycoplasma orale*	
102-3003	*Mycoplasma gallisepticum*	
102-4003	*Mycoplasma pneumoniae*	
102-5003	*Mycoplasma synoviae*	
102-6003	*Mycoplasma fermentans*	
102-7003	*Mycoplasma hyorhinis*	
102-8003	*Acholeplasma laidlawii*	
102-9003	*Spiroplasma citri*	
102-0002	*Mycoplasma* Set, all EP 2.6.7 listed species	2 vials per species, 10 CFU each

PCR Clean™ (DNA Remover™)

15-2025/15-2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 Sachets

Mycoplasma Off™

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 x 1000 ml
15-1001	Surface disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

13-0050/-0150	Contamination Prevention Reagent 100x concentrate	1000 ml/ 5 x 1000 ml

WaterShield™

15-3025/-3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

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