Venor®GeM qOneStep

Mycoplasma Detection Kit for qPCR

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

Venor®GeM qOneStep mycoplasma detection kit is designed for the direct detection of *Mollicutes*, such as *Mycoplasma* (frequently used interchangeably with *Mollicutes*), *Acholeplasma*, and *Spiroplasma*, in cell cultures, cell culture media, and other biological matrices.

TEST PRINCIPLE

The Venor®GeM qOneStep Kit is based on real-time or quantitative PCR (qPCR), as the established method of choice for rapid, robust and sensitive detection of mycoplasma contaminations.
The primer set included in the kit is designed to specifically target and amplify the highly conserved 16S rRNA coding region of the mycoplasma genome. This allows detection of *M. orale*, *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. salivarium*, and *M. hominis*, but also the less frequent strains *M. pneumoniae*, *Acholeplasma laidlawii*, *M. synoviae* and *Ureaplasma* species. Eukaryotic DNA (including human) and other bacterial DNA (except those reported in the section "Assay Characteristics") are not amplified by the Venor®GeM OneStep Kit.
The entire test requires less than 3 hours, and, in contrast to methods like luminescence-based enzyme assays, fluorescent staining, or culture methods, does not require viable mycoplasma cells. Notably, the detection by PCR is considered to be superior in terms of sensitivity and precision in comparison to several biochemical and cellular approaches.

The kit contains all necessary qPCR components including hot-start Taq polymerase, primers, and dNTPs. False-negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA, already included in the qOneStep Mix. The amplification of the Internal Control DNA is detected at 560 nm (HEX™ channel), whereas the mycoplasma-specific amplification is detected at 520 nm (FAM™ channel).

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

CONTENT

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 mix must be stored at ≤ – 18 °C.

Component	Quantity			Cap color
	25 reactions Cat. No. 11-91025	100 reactions Cat. No. 11-91100	250 reactions Cat. No. 11-91250	
qOneStep Mix	1 × (lyophilized)	4 × (lyophilized)	10 × (lyophilized)	red
Rehydration Buffer	1 × 1.8 ml	2 × 1.8 ml	5 × 1.8 ml	blue
Positive Control DNA	1 × (lyophilized)	1 × (lyophilized)	1 × (lyophilized)	green
PCR Grade Water	1 × 2.0 ml	1 × 2.0 ml	1 × 2.0 ml	white

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The Venor®GeM qOneStep kit contains necessary reagents for setting up the PCR. Additional consumables and equipment are 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, DNase- and RNase-free
- Microcentrifuge for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)

SPECIMEN

Samples should be collected when cell cultures reach 80 to 90 % confluence. Cell culture supernatants are very well suited for the mycoplasma test and do not require additional sample preparation.
However, PCR inhibiting substances may accumulate in the cell cultures medium, which will make it necessary to extract the DNA prior to the PCR test (see below for further information). Note that penicillin or streptomycin in culture media are not known to inhibit mycoplasma nor affect the test's sensitivity.

The average mycoplasma concentration in cell culture is $\sim 10^6$ particles per ml with a maximum of 10^8 particles per ml. Within this range, a sufficient amount of mycoplasma DNA is present in the supernatant for successful application of the qPCR test. Prepare the qPCR template as follows:

1.	Transfer 100 µl of cell culture supernatant to a sterile 1.5 ml reaction tube. Close the lid tightly.
2.	Incubate the sample at 95 °C for 10 min (at least 5 min).
3.	Centrifuge the sample for 30 sec at max. speed (e.g. 10,000 × g) to pellet cellular debris.
4.	Use 2 µl of the supernatant directly for qPCR, or store the sample for up to 6 days at +2 to +8 °C or at ≤ – 18 °C for long term storage.

Cell pellets cannot be used directly for the test due to the negative influence of cell debris on the PCR reaction. Cell pellets, higher PCR input volumes (> 2 µl), or other biological materials such as foetal calf serum (FCS, > 5 %), vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction prior to PCR. The Venor®GeM qOneStep assay was extensively tested with Venor®GeM Sample Preparation kit (Cat. No. 56-1010/-1050/-1200). Extracted DNA can be stored at +2 to +8 °C for up to 6 days or at ≤ – 18 °C for long-term storage.

PRECAUTIONS

Venor®GeM qOneStep 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 a suitable lab coat and disposable gloves. This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

ADDITIONAL NOTES

⇨These instructions must be understood to successfully use the Venor®GeM qOneStep Kit. The reagents supplied should not be mixed with reagents from different batches but 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 results.

⇨PCR inhibition is likely to be caused by the sample matrix. Thus, we recommend our Venor®GeM Sample Preparation kits. 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.

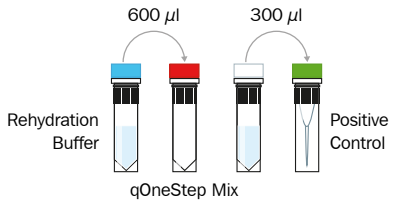
⇨Set up at least one negative control sample (non template control) in each PCR. Use the elution buffer for the NTC in case of extracted DNA.

⇨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 levels (e.g. 3 × LOD_{95}).

PROCEDURE – OVERVIEW

1. Reagent Preparation

qOneStep Mix and Positive Control

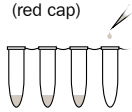


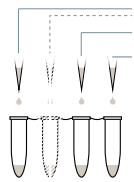
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR Reactions

loading the test tubes

aliquot 23 µl qOneStep Mix (red cap)

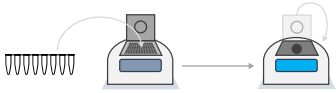




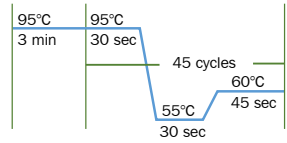
+ 2 µl sample
+ 2 µl Positive Control (green cap)
+ 2 µl fresh cell culture medium (Negative Control)

briefly

3. Start PCR amplification



Start PCR program



Rehydration Buffer
qOneStep Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

This procedure overview is not a substitute for the detailed manual.

MB_SI_Venor-GeM-qOneStep_01_EN

PROCEDURE - STEP BY STEP

The test should be carried out with negative and positive controls and samples in duplicates. All reagents and samples must be equilibrated to +2 to +8 °C prior use. After reconstitution, the reagents must be stored at ≤ -18 °C. Repeated freezing and thawing should be avoided. For small sample numbers, we recommend the preparation of aliquots of reconstituted qOneStep Mix and Positive Control DNA.

1. Reagent preparation

1.	qOneStep Mix Positive Control DNA	Red cap Green cap	Spin down all lyophilized components at max speed for 5 sec
2.	qOneStep Mix	Red cap	Add 600 µl Rehydration Buffer (blue cap) For sample kit only: Add 240 µl Rehydration Buffer
3.	Positive Control DNA	Green cap	Add 300 µl of PCR grade water (white cap)
4.	qOneStep Mix Positive Control DNA	Red cap Green cap	Incubate at room temperature for 5 min
5.	qOneStep Mix Positive Control DNA	Red cap Green cap	Vortex briefly and spin down for 5 sec

2. Preparation of PCR reactions

Follow this scheme to set up the test:

1.	Aliquot 23 µl of qOneStep Mix to each PCR tube.
2.	Negative Controls: add 2 µl fresh cell culture medium or elution buffer from DNA extraction kit (see chapter “Specimen“)
3.	Samples: add 2 µl of cell culture supernatant or DNA extract.
4.	Positive Control: add 2 µl Positive Control DNA (green cap).
5.	Close the PCR tubes tightly and spin down briefly.

3. Start qPCR amplification

1.	Place PCR tubes in the qPCR device and close the lid.
2.	Program the qPCR cycler (a technical note with detailed cycler programs of selected qPCR cyclers is available on our website www.minerva-biolabs.com).
3.	Start the program.

This assay was tested on the following qPCR devices:

qPCR device	Manufacturer
CFX96™	Bio-Rad Laboratories
LightCycler® 2.0	Roche Diagnostics
ABI Prism® 7500	Applied Biosystems
Rotor-Gene® 6000	Corbett Research
Mx3005P®	Agilent Technologies
AriaMx	Agilent Technologies

For the detailed qPCR cycler programs please visit our website www.minerva-biolabs.com

DATA INTERPRETATION

The presence of mycoplasma is indicated by an increasing fluorescence signal in the FAM™ channel. The quantification is based on threshold cycle (C_t) values and a DNA standard curve. The exact procedure for obtaining C_t 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 the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by $C_t < 40$. PCR reactions with $C_t \geq 40$ are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM™ or the HEX™ channel, or both. The mycoplasma DNA and Internal Control function as competitors in the PCR. Thus, the more mycoplasma DNA is in the sample, the higher the signal in the FAM™ channel and the lower the internal control signal in the HEX™ channel. The following table will help with the interpretation of PCR results:

Detection of Mycoplasma FAM™ channel	Internal control HEX™ channel	Interpretation
positive	irrelevant	Mycoplasma are detected in the sample
negative	negative	PCR inhibition
negative	positive	No mycoplasma are detected in the sample

ASSAY CHARACTERISTICS

For EP 2.6.7 compliant lot release testing of biopharmaceuticals please consider the product versions Venor®GeM qEP for real-time qPCR or Venor®GeM Classic for conventional PCR.
The kit cannot detect any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus*, and *Streptococcus*, as well as *Burkholderia*. The assay can detect *Staphylococcus epidermidis*. The table below shows a selection of the most relevant species that can be (Positive) and those that cannot be detected (Negative: other microorganisms, including bacteria and eukaryotic samples).

Positive (Mollicutes)	**Negative**	
	Bacteria	**Mammals**
Acholeplasma laidlawii	*Clostridium acetobutylicum*	Vero-B4
Mycoplasma hyorhinis	*Lactobacillus acidophilus*	Per.C6
Mycoplasma fermentans	*Streptococcus pneumoniae*	RK13
Mycoplasma orale		CHO-K1
Mycoplasma synoviae		Murine genomic DNA
Mycoplasma pneumoniae		Calf thymus DNA
Mycoplasma arginini		Foetal bovine serum
Mycoplasma gallisepticum		
Spiroplasma citri		
Mycoplasma arthritidis		
Mycoplasma genitalium		
Mycoplasma hominis		
Mycoplasma penetrans		
Mycoplasma salivarium		
Ureaplasma urealyticum		

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or im-plied, 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

Contamination Control Kits for conventional PCR

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

Contamination Control Kits for qPCR

11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 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

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*
102-1103	*Mycoplasma salivarium*
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 / JP listed species 2 vials per species, 10 CFU each

100CFU™ Sensitivity Standards, 3 vials with 100 CFU each, 2 vials negative control

103-1003	*Mycoplasma arginini*
103-2003	*Mycoplasma orale*
103-3003	*Mycoplasma gallisepticum*
103-4003	*Mycoplasma pneumoniae*
103-1103	*Mycoplasma salivarium*
103-5003	*Mycoplasma synoviae*
103-6003	*Mycoplasma fermentans*
103-7003	*Mycoplasma hyorhinis*
103-8003	*Acholeplasma laidlawii*
103-9003	*Spiroplasma citri*

PCR Cycler Validation

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

PCR Clean™

15-2025/-2500	DNA Decontamination Reagent, spray bottle/refill canister	250 ml/5 l
15-2001/-2002	DNA Decontamination Reagent, Wipes in dispenser box/refill box	50 wipes/ 5×50 wipes

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant Spray, spray bottle/canister	1 l/5l
15-1001/-5001	Surface Disinfectant Wipes in dispenser box/refill pack	50 wipes/5×50 wipes

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/3× 50 ml

WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200× concentrate	15× 10 ml/3× 50 ml/500 ml

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