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

INTENDED USE

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 qOneStep 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 °C to +8 °C until use. After reconstitution, the reagents must be stored at ≤-18 °C for a maximum of 12 months, but the expiry date must not be exceeded. Repeated thawing and freezing should be avoided.

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 (preferably low-bind)
- Microcentrifuge for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10 µl, 100 µl, 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 ~ 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 to 500 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 s 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 °C 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 vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction prior to PCR. Serum in the cell culture medium can have inhibitory effects on the PCR. As the serum concentration in the cell culture medium increases, the probability ob PCR inhibition also increases. DNA extraction prior to PCR might be necessary to avoid false negative results.

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 °C 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.

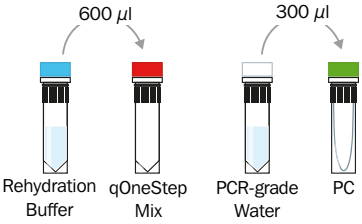
ADDITIONAL NOTES

- These instructions must be understood to successfully use the Venor®GeM qOneStep kit. The reagents supplied should not be mixed with reagents from different LOTs 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 (No Template Control, NTC) 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. $3 \times LOD_{95}$).

PROCEDURE – OVERVIEW

1. Reagent preparation

qOneStep Mix
Positive Control DNA
briefly



briefly
≥5 min RT

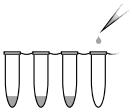
qOneStep Mix:
briefly
for 5 s

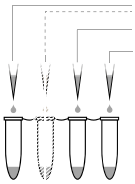
PC:
thoroughly
for 5 s

2. Preparation of PCR reactions

Loading the test tubes

Aliquot 23 µl qOneStep Mix

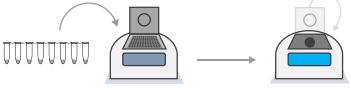




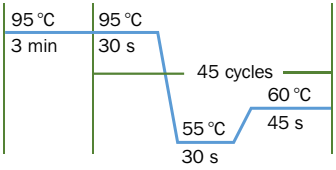
+ 2 µl Sample
+ 2 µl PC
+ 2 µl Fresh cell cultur medium or elution buffer for extracted DNA (NTC)

briefly

3. Start PCR amplification



Start PCR program



Rehydration Buffer
qOneStep Mix
PCR-grade Water
Positive Control DNA (PC)

vortex
centrifuge
add
incubate

NTC = No Template Control

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

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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 °C to +8 °C prior use. 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 s.
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	Vortex briefly and incubate at least for 5 min at room temperature.*
5.	qOneStep Mix	Red cap	Vortex briefly and spin down for 5 s.
6.	Positive Control DNA	Green cap	Vortex thoroughly and spin down for 5 s.

* Increasing the incubation temperature of the Positive Control DNA to 60 °C can have a positive effect on the final result.

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
LightCycler® 480	Roche Diagnostics
ABI Prism® 7500	Applied Biosystems
ABI StepOne™/ Plus™	Applied Biosystems
Rotor-Gene® 6000	Corbett Research
Mx3005P®	Agilent Technologies
AriaMx	Agilent Technologies
QuantStudio™ 3/5	Thermo Fisher Scientific

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 (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 the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by Ct <40. PCR reactions with Ct ≥ 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 DNA 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 Mollicutes 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

Mycoplasma detection kits for conventional PCR		
11-1025/-1050/-1100/-1250	Venor®GeM Classic	25/50/100/250 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance	24/48/96/240 reactions
11-8025/-8050/-8100/-8250	Venor®GeM OneStep	25/50/100/250 reactions
Mycoplasma detection kits for qPCR		
11-9025/-9100/-9250	Venor®GeM qEP	25/100/250 reactions
Bacteria detection kit for conventional PCR		
12-1025/-1100/-1250	Onar® Bacteria	25/100/250 reactions
Sample Preparation		
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-3010/3100	Venor® Mycoplasma Extraction	10/100 extractions
Mycoplasma Elimination		
10-0200/0500/1000	Mynox®	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold	2/5/10 treatments
10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control		
102-XXXX	Various mycoplasma species (See Minerva Biolabs website for available species)	
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-XXXX	Various mycoplasma species (See Minerva Biolabs website for available species)	
PCR cycler verification		
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
Mycoplasma Off™		
15-1000/-5000	Surface disinfectant, spray bottle/refill canister	1 l/5 l
15-1001	Surface disinfectant, wipes in a dispenser box	50 wipes
15-5001	Surface disinfectant, wipes in refill bags	5 × 50 wipes
PCR Clean™		
15-2025/-2200/-2500	DNA decontamination reagent, spray bottle/refill bottles	250 ml/4 × 500 ml/5 l
15-2001	DNA decontamination reagent, wipes in a dispenser box	50 wipes
15-2002	DNA decontamination reagent, wipes in refill bags	5 × 50 wipes
ZellShield®		
13-0050/-0150	Contamination prevention reagent 100 × concentrate	50 ml/ 3 x 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
Residual DNA Testing		
58-0121/-0122	MiQuant® Residual DNA HEK-293 – dPCR	35/140 µl primer/probe mix
58-0221	dPCR Validation Standard HEK-293	1 vial

Minerva Biolabs GmbH
Schkopauer Ring 13
D-12681 Berlin, Germany

www.minerva-biolabs.com
Ordering: order@minerva-biolabs.com
Support: support@minerva-biolabs.com

USA & Canada

Minerva Biolabs Inc.
400 Route 518
Skillman, NJ 08558-2401, USA

www.minervabiolabs.us
Ordering: order@minervabiolabs.us
Support: help@minervabiolabs.us

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