# 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 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. The rehydrated mix must be stored at ≤-18 °C for a maximum of 12 month, 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 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 °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 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 °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 (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 - 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. After reconstitution, the reagents must be stored at ≤-18 °C for a maximum of 12 month, but the expiry date must not be exceeded. Repeated thawing and freezing should be avoided. 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<br>Positive Control DNA | Red cap<br>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)<br>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<br>Positive Control DNA | Red cap<br>Green cap | Incubate at room temperature for 5 min |
| 5. | qOneStep Mix<br>Positive Control DNA | Red cap<br>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 (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 $\geq 40$ 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

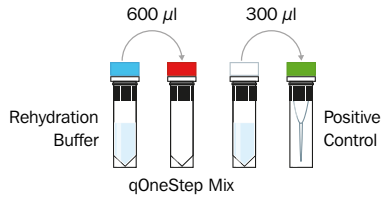
For EP 2.6.7 compliant lot release testing of biopharmaceuticals please consider the product versions Venor® GeM qEP for real-time qPCR or Venor® GeM Classic for conventional PCR.
The kit cannot detect any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus*, and *Streptococcus*, 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* | | |

## PROCEDURE – OVERVIEW

### 1. Reagent preparation

qOneStep Mix and Positive Control

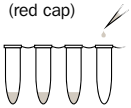


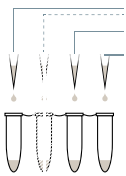

for 5 min RT
briefly
for 5 sec

### 2. Preparation of PCR reactions

loading the test tubes

aliquot 23 µl qOneStep Mix (red cap)

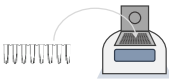




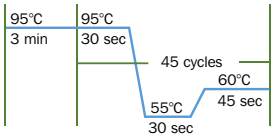
+ 2 µl sample
+ 2 µl Positive Control (green cap)
+ 2 µl fresh cell culture medium (Negative Control)

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.

## 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.

*Trademarks*

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registered trademark of Applera Corporation or its subsidiaries in the US and certain other countries. CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. Rotor-Gene is a registered trademark of the Qiagen Group. Mx3005P is a registered trademark of Stratagene, an Agilent Technologies company. StepOne and StepOnePlus are trademarks of Applied Biosystems or its subsidiaries in the U.S. and/or certain other countries. FAM and HEX are trademarks of Applera Corp. or its subsidiaries.

Venor, Mynox, Onar, and ZellShield are registered trademarks and PCR Clean, Mycoplasma Off, 10CFU, 100CFU, PCR Cycler Check, and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.

## 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 |
| 56-3010/3100 | Venor® GeMSP Kit-Beads | 10/100 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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