Venor® GeM OneStep

Mycoplasma Detection Kit for conventional PCR

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

SYMBOLS

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The Venor® GeM OneStep kit is designed for the detection of mollicutes, such as mycoplasma (frequently used interchangeably with mollicutes), acholeplasma, and spiroplasma, in cell cultures and other biological matrices.

TEST PRINCIPLE

The Venor® GeM OneStep kit is based on conventional (or endpoint) PCR, 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, M. hominis,* usually encountered as contaminants in cell cultures, as well as *M. pneumoniae, Acholeplasma laidlawii, M. synoviae, Spiroplasma citri and Ureaplasma* species. Depending on the mycoplasma species, the amplicon is in the 265-278 bp size range (see also "Data Interpretation"). Eukaryotic (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 approach.

The kit contains all necessary components for conventional PCR. The lyophilized OneStep Mix includes hot-start Taq polymerase, primers, and dNTPs. The mix also contains an Internal Control DNA to verify whether the PCR reaction took place without any inhibition. In case of successful PCR, the Internal Control DNA gives rise to a 191 bp amplicon (see also „Data Interpretation"). The provided lyophilized Positive Control DNA (dissolved as described in the „Procedure" sections) can be used to check the full functionality of the detection assay. The Internal Control DNA as well as the Positive Control DNA are essential tools to assess the assay performance.
Once dissolved in the given buffer, the OneStep Mix only needs to be aliquoted into reaction tubes before samples are added, prior to PCR. The OneStep 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 result is minimized. Please note that UNG is not included in the Venor® GeM OneStep kit.

CONTENT

Each kit contains reagents for 25, 50, 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 until the expiry of the labelled shelf life. Repeated thawing and freezing should be avoided.

Component	Quantity				Cap Color
	25 reactions Cat. No. 11-8025	50 reactions Cat. No. 11-8050	100 reactions Cat. No. 11-8100	250 reactions Cat. No. 11-8250	
OneStep Mix	1 vial lyophilized	2 vials lyophilized	4 vials lyophilized	10 vials lyophilized	red
Rehydration Buffer	1 vial 1.3 ml	1 vial 1.3 ml	2 vials 1.3 ml	5 vials 1.3 ml	blue
Positive Control DNA	1 vial lyophilized	1 vial lyophilized	1 vial lyophilized	1 vial lyophilized	green
PCR grade Water	1 vial 2.0 ml	1 vial 2.0 ml	1 vial 2.0 ml	1 vial 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 OneStep kit contains necessary reagents for setting up the PCR. Additional consumables and equipment are supplied by the user:

- PCR cycler
- PCR reaction tubes (preferably low-bind) or plates, DNA- and nuclease-free recommended by the manufacturer of your thermal cycler
- Pipettes with corresponding filter tips (10 µl, 100 µl, and 1000 µl)
- Microcentrifuge for 1.5 ml and 2 ml reaction tubes
- Agarose gel electrophoresis system including dye, marker, and loading buffer
- 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 culture medium, which will make it necessary to extract the DNA prior to PCR test (see further information below). Note that penicillin or streptomycin in the culture media are not known to inhibit mycoplasma nor affect the test's sensitivity.

The average mycoplasma titer 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 PCR test. Prepare the PCR template as follows:

1.	Transfer 100 µl to 500 µl of cell culture supernatant to a sterile 1.5 ml reaction tube. Close the lid tightly.
2.	Incubate the sample supernatant at 95 °C for 10 minutes (at least 5 minutes).
3.	Centrifuge the sample for 15 sec at max. speed (e.g. 10,000 × g) to pellet cellular debris.
4.	Use 2 µl directly for PCR, 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 sample volumes (>2 µl), or other biological materials such as vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction prior to PCR. Foetal calf serum (FCS) content (>5%) in samples is known to increase the probability of PCR inhibition. DNA extraction prior to PCR might be necessary for these samples to avoid false negative results.

The Venor® GeM OneStep kit was tested with Venor® GeM Sample Preparation Kit (Cat. No. 56-1010/56-1050/56-1200). 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 OneStep kit is for research use only and should not be used for clinical diagnostics or testing of human samples. 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.

Performing the tests according to good laboratory practice helps avoiding carry-over contaminations and false positive results and, ultimately, helps obtaining reliable results.

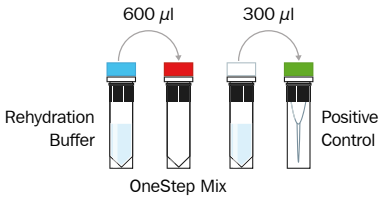
ADDITIONAL NOTES

- These instructions must be understood to successfully use the Venor® GeM OneStep 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 or, in case of extracted DNA, by the elution buffer. Thus, we recommend our Venor® GeM Sample Preparation Kit. Any other DNA extraction kit needs to be qualified.
- It is important to include positive and negative controls on a regular basis to monitor the reliability of your results and in case of troubleshooting. Include min. 1 no template control (NTC, negative control) per PCR, by using fresh cell culture medium or elution buffer (in case of extracted DNA).
- Avoid carry-over contaminations by preparing the positive controls after the negative controls and test reactions.
- Rehydrate the OneStep Mix (as indicated in the section „Procedure“) and aliquot the required volume for each reaction directly into PCR reaction tubes or strips. Use immediately or freeze the rehydrated PCR mix.
- The control samples must be processed in the same manner as the test samples.

PROCEDURE – OVERVIEW

1. Reagent preparation

OneStep Mix and Positive Control

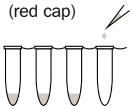


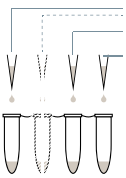
for 5 min RT
briefly
for 5 sec

2. Preparation of PCR reactions

loading the test tubes

aliquot 23 µl OneStep Mix (red cap)





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

briefly

3. Start PCR reaction

1 cycle	94 °C	for 2 min
39 cycles	94 °C	for 30 sec
	55 °C	for 30 sec
	72 °C	for 30 sec
hold 4	to 8 °C	

100 bp DNA Ladder
Negative Control
Positive Control
Inhibited sample
Negative sample
Positive sample, weak contamination
Positive sample, strong contamination

Rehydration Buffer
OneStep Mix
PCR grade water
Positive Control

incubate
vortex
centrifuge
add

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

PROCEDURE - STEP BY STEP

⇨ Set up all samples in duplicates.

1. Reagent preparation

1.	OneStep Mix Positive Control DNA	Red cap Green cap	Centrifuge all lyophilized components at max. speed for 5 sec
2.	OneStep Mix	Red cap	Add 600 µl Rehydration Buffer (blue cap) For sample kit only: Add 120 µl Rehydration Buffer
3.	Positive Control DNA	Green cap	Add 300 µl of PCR grade Water (white cap)
4.	OneStep Mix Positive Control DNA	Red cap Green cap	Incubate at room temperature for 5 min
5.	OneStep Mix Positive Control DNA	Red cap Green cap	Vortex and spin down for 5 sec

After reconstitution, the reagents must be stored at ≤-18 °C until the expiry of the labelled shelf life. Repeated thawing and freezing should be avoided. For small sample numbers, we recommend preparing aliquots of reconstituted OneStep Mix and the Positive Control DNA.

2. Preparation of PCR reactions

Follow this scheme to set up the test:

1.	Aliquot 23 µl of OneStep Mix to each PCR tube.	
2.	Negative Controls:	add 2 µl fresh cell culture medium or elution buffer from DNA extraction kit (see section“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.	

3. Start PCR amplification

1.	Place the PCR tubes in the cycler and close the lid tightly.	
2.	Program the PCR cycler or check stored temperature profiles. 1 cycle 39 cycles Hold between +4 °C and +8 °C	 94 °C for 2 min 94 °C for 30 sec 55 °C for 30 sec 72 °C for 30 sec
3.	Start the program.	

4. Agarose gel electrophoresis

1.	Cast a 1.5 to 2% agarose gel including an appropriate DNA stain (max. 5 mm thick, 5 mm comb).
2.	Mix 5 µl from each PCR reaction with a bromophenol blue loading buffer and load the mix. Note: Bromophenol blue will run similarly to ~270 bp PCR fragments and may therefore mask the PCR product. Make sure to use bromophenol blue in a low concentration or other dyes such as Orange G or Xylene Cyanol.
3.	Perform the gel electrophoresis (e.g. 20 min at 100 V, depending on your gel electrophoresis apparatus).
4.	Visualize the PCR results on a suitable transilluminator. Expected amplicon sizes: Internal control 191 bp *Mycoplasma spp.* 265 – 278 bp

DATA INTERPRETATION

The Internal Control DNA gives rise to a distinct 191 bp band in every lane indicating a successfully performed PCR. Due to competition between the internal control and the target reaction, this band will fade out when large amounts of primary target are initially present (e.g. mycoplasma DNA input of $>10^3$ copies per PCR). The initial concentration of Positive Control DNA is higher than 10^3 copies per PCR. Consequently, the internal control is usually not visible in the positive control reaction.

Other PCR products may be visible in the gel as faint, diffuse bands of different sizes (neither 191 bp nor ~270 bp). This does not indicate positive results. These products are unspecific and caused by non-specific annealing (e.g. high DNA input of >100 µg/ml). Also, primer self-annealing may give rise to a band of 80–90 bp in size. This again does not affect the sensitivity and precision or results of the test.

If the PCR test shows inhibition due to sample matrix (lower band intensity compared to negative control), DNA extraction needs to be performed prior to re-testing the sample (see section „Specimen“).

Detection of mycoplasma band at ~270 bp	**Internal Control DNA band at 191 bp**	**Interpretation**
Positive	Irrelevant	Mycoplasma DNA present in the sample
Negative	Negative	PCR inhibition
Negative	Positive	No mycoplasma DNA detectable in the sample

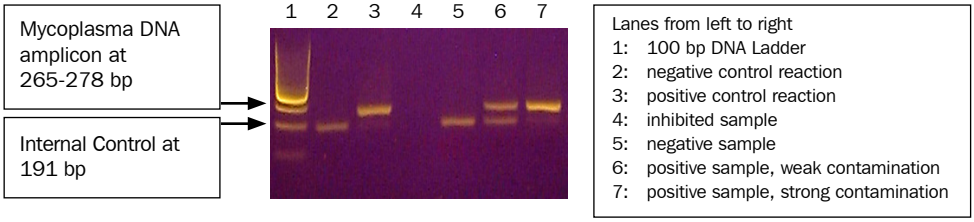


Fig. 1: A typical agarose gel image

ASSAY CHARACTERISTICS

Sensitivity

The detection limit was determined at approx. 20 genome copies/PCR reaction for different mycoplasma species. Due to the small sample volume of 2 µl, this kit does not comply with the detection limit of 10 CFU/ml according to *European Pharmacopoeia* (EP) 2.6.7. For EP 2.6.7 compliant lot release testing of biopharmaceuticals please consider the product versions Venor® GeM Classic for conventional PCR or Venor® GeM qEP for real-time qPCR.

Specificity

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 EP 2.6.7 listed bacteria and eukaryotic samples) by the assay. Unspecific PCR products such as faint, diffuse bands of different sizes are rarely observed (see also section "Data Interpretation"). The kit does not detect any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus,* and *Streptococcus*. Likewise, the waterborne germ *Burgholderia* is not detected. The assay can detect *Staphylococcus epidermidis* at concentrations above 10^4 genome copies/µl.

A large number of mollicutes sequences have been published. The primers of the kit were aligned against the NCBI data and scrutinized for homologies within the target region of the 16S rRNA. At least 1 Ureaplasma, 7 Acholeplasma and 85 Mycoplasma show highly relevant sequence homologies and are presumably detected as positive.

Positive (Mollicutes)	**Negative**		
	EP 2.6.7 listed bacteria	**Other microorganisms**	**Mammals**
Acholeplasma laidlawii	*Clostridium acetobutylicum*	*Chlamydia trachomatis*	Vero-B4
Mycoplasma arginini	*Lactobacillus acidophilus*	*Legionella pneumophila*	Per.C6
Mycoplasma arthritidis	*Streptococcus pneumoniae*	*Micrococcus luteus*	RK13
Mycoplasma fermentans		*Candida albicans*	CHO-K1
Mycoplasma gallisepticum		*Enterococcus faecalis*	*Murine genomic DNA*
Mycoplasma genitalium		*Enterobacter aerogenes*	*Calf thymus DNA*
Mycoplasma hominis		*Escherichia coli*	*Foetal bovine serum*
Mycoplasma hyorhinis		*Proteus mirabilis*	
Mycoplasma orale		Bacillus cereus	
Mycoplasma penetrans			
Mycoplasma pneumoniae			
Mycoplasma salivarium			
Mycoplasma synoviae			
Spiroplasma citri			
Ureaplasma urealyticum			

APPENDIX

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 the use, the results of use, or the inability to use this product.

Trademarks
Venor, Mynox, Onar and ZellShield are registered trademarks and PCR Clean, 10CFU, 100CFU, Mycoplasma Off 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
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
11-91025/-91100/-91250	Venor® GeM qOneStep 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
PCR Quantification Standards, 1 x 10^8 genomes / vial		
52-XXXX	Various genomes (See Minerva Biolabs website for available genomes)	
10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control		
102-XXXX	Various mycoplasma species (See Minerva Biolabs website for available mycoplasma species)	
102-0002	Mycoplasma Set, all EP 2.6.7 listed species, 2 vials per species, 10 CFU each	
100CFU™ Sensitivity Standards, 3 vials with 100 CFU each, 2 vials negative control		
103-1003	Various mycoplasma species (See Minerva Biolabs website for available mycoplasma species)	
PCR Clean™		
15-2025/-2500	DNA Decontamination Reagent, Spray bottle/canister	250 ml/5 l
15-2001	DNA Decontamination Reagent, Wipes in a dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes in refill packs	5 × 50 wipes
Mycoplasma Off™		
15-1000/-5000	Surface Disinfectant, Spray bottle/canister	1 l/5 l
15-1001	Surface Disinfectant, Wipes in a dispenser box	50 wipes
15-5001	Surface Disinfectant, Wipes in refill packs	5 × 50 wipes
ZellShield®		
13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/5 × 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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