Venor®GeM OneStep

Mycoplasma Detection Kit for conventional PCR

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Order No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

Venor®GeM OneStep mycoplasma detection kit is designed for the direct detection of mycoplasma contaminations in cell cultures, cell culture media, and other biological matrices.

TEST PRINCIPLE

The Venor®GeM OneStep assay is based on PCR amplification, as the established method of choice for the rapid, robust and sensitive detection of mycoplasma contamination. The assay targets a highly conserved region within the mycoplasma genome to detect *M. orale*, *M. hyorhinis*, *M. arginini*, *M. fermentans*, *M. salivarium*, and *M. hominis*, as prevalent cell culture contaminants, and also other less frequent strains, such as *M. pneumoniae*, *Acholeplasma laidlawii*, *M. synoviae* and *Ureaplasma* species (see list of detected mycoplasma in section "Assay Characteristics"). With this kit, you are able to detect all mycoplasma species in a single experiment, whereas eukaryotic and bacterial DNA is not amplified. The procedure takes less than 3 hours, and, in contrast to other 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.

Mycoplasma are specifically detected by amplifying the 16S rRNA coding region in the mycoplasma genome. Depending on the mycoplasma species, the amplicon is ~ 270 bp in size. The kit contains all necessary PCR components including hot-start Taq polymerase, primers, and dNTPs. The Internal Control DNA and the Positive Control DNA are means to assess the assay's performance. The Internal Control DNA gives rise to a 191 bp amplicon.

The OneStep Mix 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 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 to +8 °C until use. The rehydrated mix must be stored at < – 18 °C.

Component	Quantity				Cap Color
	25 reactions Order No. 11-8025	50 reactions Order No. 11-8050	100 reactions Order No. 11-8100	250 reactions Order No. 11-8250	
OneStep Mix	1 × (lyophilized)	2 × (lyophilized)	4 × (lyophilized)	10 × (lyophilized)	red
Rehydration Buffer	1 × 1.3 ml	1 × 1.3 ml	2 × 1.3 ml	5 × 1.3 ml	blue
Positive Control DNA	1 × (lyophilized)	1 × (lyophilized)	1 × (lyophilized)	1 × (lyophilized)	green
Tris/EDTA Buffer	1 × 2.0 ml	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).

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 reaction tubes and caps
- PCR cycler
- Microcentrifuge for 1.5 ml and 2 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)

SPECIMEN

Samples should be obtained from cell cultures with 80 to 90 % confluence. 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, making 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 number 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 successfully applying the PCR test. Prepare the PCR template as follows:

1.	Transfer 100 µl of the supernatant from the cell culture to a sterile reaction tube. Close the lid tightly.
2.	Incubate the sample supernatant at 95 °C for 5 minutes.
3.	Centrifuge the sample at max. speed briefly (15 s) to pellet any cellular debris.
4.	Use 2 µl directly for PCR, 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 cell debris that will interfere with the PCR reaction. However, cell pellets as well as foetal calf serum, vaccines, cryo stocks, and paraffin-embedded samples require DNA extraction in advance. The OneStep assay was qualified with these DNA extraction kits:

- Venor®GeM Sample Preparation kit (Order No. 56-1100) for manual DNA extraction, or
- Venor®GeM Sample Preparation Kit—IP C16 (Order No. 56-2096) for automated DNA extraction.

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 OneStep kit is for research 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.

Cross contaminations may lead to false-positive test results. Thus, all tests should be performed according to good laboratory practice.

IMPORTANT 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 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.

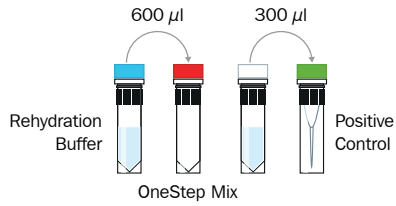
⇨ Set up at least one negative control sample (non template control) in each PCR. Use elution buffer for the NTC in case of extracted DNA.

⇨ 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 level (e.g. 3x LOD_{95}). Please note that Minerva Biolabs also offers to participate in external quality control programs.

PROCEDURE – OVERVIEW

1. Reagent Preparation

OneStep Mix and Positive Control

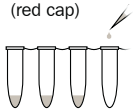


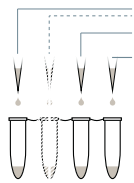
for 5 min RT
briefly
for 5 sec

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
Tris/EDTA Buffer
Positive Control

incubate
vortex
centrifuge
add

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

PROCEDURE

⇨ Set up all samples in duplicates.

⇨ Equilibrate samples and reagents to + 2 – +8 °C.

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 Tris/EDTA Buffer (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 DNA briefly and spin for 5 sec

After reconstitution, the reagents must be stored at < – 18 °C. Repeated freeze-thaw-cycles should be avoided. For small sample numbers, we recommend to prepare 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 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.	

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 94 °C for 2 min 39 cycles 94 °C for 30 sec 55 °C for 30 sec 72 °C for 30 sec Hold from 4 °C to 8 °C	
3.	Start the program	

4. Agarose gel electrophoresis

1.	Cast a 1.5 % 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		
3.	Note: Bromophenol blue in a low concentration should be used as loading dye.		
4.	Result reading:	Internal control Mycoplasma spp.	191 bp 265 – 278 bp

INTERPRETATION OF RESULTS

The Internal Control DNA will give rise to a distinct 191 bp band in every lane indicating a successfully performed PCR. This band will fade out with increased amounts of primary target amplification (e.g. mycoplasma DNA input of $> 5 \times 10^6$ copies per ml). The initial concentration of positive control DNA is higher than 5×10^6 copies per ml to account for DNA loss due to repeated freeze-thaw cycles. 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 the sample matrix (lower band intensity compared to negative control), DNA extraction needs to be performed prior to re-testing the sample (see chapter „Specimen“).

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

Fig. 1: A typical agarose gel image

100 bp DNA Ladder

Negative control

Positive control

Inhibited sample

Negative sample

Positive sample, weak contamination

Positive sample, strong contamination

ASSAY CHARACTERISTICS

1. Sensitivity

The detection limit depends on the species. The lower detection limit (cut-off limit) was found below 20 genomes / PCR for all tested species:

Species	Lower Limit [genomes/PCR]
Acholeplasma laidlawii	< 20
Mycoplasma fermentans	< 10
Mycoplasma synoviae	< 20
Mycoplasma pneumoniae	< 20
Spiroplasma citri	< 20

2. Specificity

Cross-reactivity with eukaryotic DNA origin could not be found. Unspecific PCR products such as faint diffuse bands are rarely observed. (see chapter "Interpretation of Results"). The kit does not detect any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus* and *Streptococcus*. Likewise, the waterborne germ *Burkholderia* is not detected. The following species were positively tested with Venor®GeM OneStep:

Species	Amplicon Size [bp]	Species	Amplicon Size [bp]
Mycoplasma orale	266	*Mycoplasma bovis*	267
Mycoplasma pneumoniae	273	*Mycoplasma cloacale*	266
Mycoplasma penetrans	274	*Mycoplasma hyosynoviae*	265
Mycoplasma pirum	274	*Mycoplasma synoviae*	266
Acholeplasma laidlawii	273	*Mycoplasma salivarium*	266
Mycoplasma fermentans	267	*Mycoplasma faucium*	265
Mycoplasma hyorhinis	268	*Mycoplasma hominis*	266
Mycoplasma pulmonis	268	*Mycoplasma genitalium*	273
Mycoplasma falconis	268	*Mycoplasma bovigenitalium*	267
Mycoplasma arthritidis	267	*Mycoplasma caprine*	267
Mycoplasma arginini	267	*Mycoplasma agalactica*	267
Mycoplasma spermatophilum	267	*Mycoplasma timone*	266
Mycoplasma opalescens	266	*Spiroplasma citri*	268
Mycoplasma primatum	267		

A substantial number of Mycoplasma sequences have been published. The primers of the kit were aligned against the NCBI data base and scrutinized for homologies within the target region of the 16S rRNA. The following table shows all mycoplasma species with relevant sequence homologies, and, thus, presumptively positive PCR results:

M. agassizii	*M. caviae*	*M. felifaucium*	*M. indiense*	*M. spumans*
M. alkalescens	*M. citelli*	*M. gallinaceum*	*M. iners*	*M. sualvi*
M. anseris	*M. collis*	*M. gallinarum*	*M. lagogenitalium*	*M. subdolum*
M. bovirhinis	*M. columbinasale*	*M. gallopavonis*	*M. lipofaciens*	*M. testudineum*
M. buccale	*M. columbinum*	*M. gateae*	*M. lipophilum*	*M. turnidae*
M. buteonis	*M. columborale*	*M. glycophilum*	*M. meleagridis*	*M. verecundum*
M. californicum	*M. cynos*	*M. gypis*	*M. moatsii*	
M. canadense	*M. edwardii*	*M. hyopharyngis*	*M. simbae*	
M. capricolum	*M. equirhinis*	*M. iguanae*	*M. sphenisci*	

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

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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
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 tests

Contamination Control Kits for qPCR

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

Sample Preparation

56-1050	Venor®GeM Sample Preparation Kit	50 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, 1x10^8 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™ (formerly DNA Remover™)

15-2025/15-2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4× 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 × 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 × 1000 ml
15-1001	Surface disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 × 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield®

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

WaterShield™

15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200x concentrate	30 x 5 ml/3 x 50 ml/500 ml

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