Venor®GeM Advance

Pre-aliquoted 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 Advance 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 Advance 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 Advance kit.

The entire test requires less than 3 hours, and, in contrast to methods like luminescence-based enzyme as-says, 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 provided PCR reaction tubes are pre-loaded with lyophilized PCR mix including Taq polymerase, nucleotides, primers, internal control DNA and loading dye to significantly reduce the hands-on time. The pre-loaded PCR tubes also contain 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.

Upon resuspension of the pre-dispensed PCR mix and sample loading, the strips of tubes are ready for PCR. Hence, the PCR products can be directly loaded on the agarose gel.

The kit 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 Advance kit.

CONTENT

Each kit contains reagents for 24, 48, 96 or 240 reactions. The expiry date of the unopened package is marked on the package label. The kit components should be stored until use at +2 °C to +8 °C. After rehydrating, the components should be used as soon as possible.

Component	Description	Quantity			
		24 Reactions Cat. No. 11-7024	**48 Reactions Cat. No. 11-7048**	**96 Reactions Cat. No. 11-7096**	**240 Reactions Cat. No. 11-7240**
Test Reaction Tubes	Pre-loaded and lyophilized Primer sets, Nucleotides, Polymerase, Internal Control DNA, Gel Loading buffer in 0.2 ml PCR reaction tubes	3 strips of 8 clear tubes, each	6 strips of 8 clear tubes, each	12 strips of 8 clear tubes, each	30 strips of 8 clear tubes, each
Positive Control Reaction Tubes	Pre-loaded and lyophilized Primer sets, Nucleotides, Polymerase, Internal Control DNA, Positive Control DNA in 0.2 ml PCR reaction tubes	1 strip of 8 red tubes	2 strips of 8 red tubes, each	3 strips of 8 red tubes, each	5 strips of 8 red tubes, each
Rehydration Buffer		1 vial, 1.6 ml	1 vial, 1.6 ml	2 vials, 1.6 ml	5 vials, 1.6 ml
Caps for Reaction Tubes		4 strips of 8 tubes, each	8 strips of 8 tubes, each	15 strips of 8 tubes, each	35 strips of 8 tubes, each

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 Advance kit contains all necessary components for setting up the PCR reaction. Additional consumables and equipment are supplied by the user:

- PCR cycler
- Pipettes with corresponding filter tips (10 μl and 100 μl)
- Microcentrifuge for 1.5 ml and PCR reaction tubes
- Agarose gel electrophoresis system including DNA stain
- 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 ~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 to 500 µl of cell culture supernatant from the test culture to a 1.5 ml reaction tube. Note: make sure the lid is closed tightly or sealed, if necessary, to prevent opening during heating.
2.	Incubate the sample at 95 °C for 10 minutes (at least 5 minutes).
3.	Briefly centrifuge (15 sec) the sample at approx. 13,000 rpm to pellet cellular debris.
4.	Use 2 µl of the supernatant directly for PCR.

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 Advance 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

The Venor®GeM Advance 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.

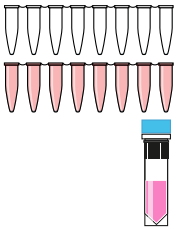
This kit does not contain hazardous substances. Remnants can be discarded according to local regulations. Performing the tests according to good laboratory practice helps avoiding carry-over contaminations and false positive results and, ultimately, helps obtaining reliable results.

ADDITIONAL NOTES

- These instructions must be understood to successfully use the Venor®GeM Advance kit. The components 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 validated.
- It is important to include positive and negative controls to monitor the reliability of your results as well as 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.
- The control samples must be processed in the same manner as the test samples.
- The appearance of the pellets in the PCR tubes may vary for technical reasons, which have no effect on the outcome of the test.

PROCEDURE – OVERVIEW

1. Components



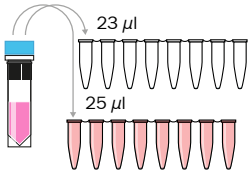
Test Reaction tubes

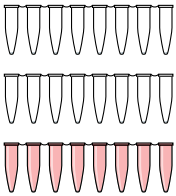
Positive Control tubes

Rehydration Buffer

PCR tubes and
Rehydration Buffer

2. Preparation of PCR Reactions





+ 2 µl fresh cell culture medium (Negative Control)
or
+ 2 µl cell culture supernatant (sample)

no addition of reagents

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

centrifuge

\+ add

storage 2-8 °C

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

PROCEDURE - STEP BY STEP

Set up negative (NTCs) and positive controls with each test.

1. Component preparation

1.	Remove required number of PCR tubes from the kit (cut tubes from strips if necessary) and close bags with remaining tubes properly.
2.	Spin down PCR tubes and Rehydration Buffer.
3.	Peel off the sealing film from the PCR tubes and add 23 µl of Rehydration Buffer to each tube. Note: Rehydrate Positive Control Tubes with 25 µl of Rehydration Buffer.

2. Add samples

Follow the pipetting sequence and close tubes.

1.	Negative Controls:	add 2 µl fresh cell culture medium or elution buffer supplied with the DNA extraction kit
2.	Samples:	add 2 µl of cell culture supernatant or DNA extract.
3.	Positive Controls:	no addition of reagents necessary
4.	Close tightly and spin all PCR tubes briefly, load the PCR cycler and start the program.	

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 between +4 °C and +8 °C
3.	Start the program

4. Agarose gel electrophoresis

1.	Cast a 1.5% to 2% agarose gel including DNA stain (max. 5 mm thick, 5 mm comb).
2.	Load 5 µl of each PCR product. No loading dye is required.
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 265 – 278 bp	Internal Control 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

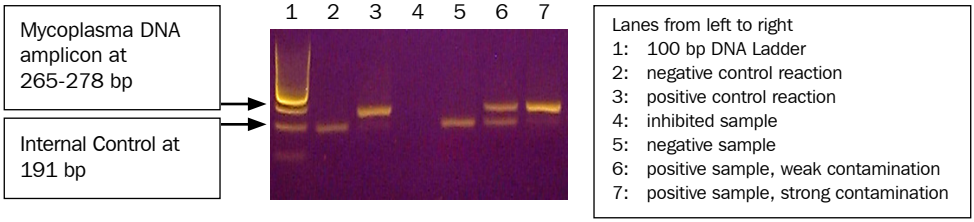


Fig. 1: A typical agarose gel image

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

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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 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
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
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 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
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
ZellShield®		
13-0050/-0150	Contamination Prevention Reagent 100 × concentrate	50 ml/3 × 50 ml
WaterShield™		
15-3025/-3075	Water Disinfection Additive for incubators and water baths, 200 × concentrate	15 × 10 ml/3 × 50 ml/500 ml
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	
Food and Water Assays		
370-1025/-1100	Meat ID™ Halal	25/100 reactions
370-2025/-2100	Vegan Control™	25/100 reactions
33-2025/-2100/-2250	AquaScreen® *Legionella* species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® *Legionella pneumophila*	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® *Pseudomonas aeruginosa*	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® *Escherichia coli*	25/100/250 reactions
PCR Cycler Validation		
57-2102	PCR Cycler Check™ Advance	6 strips, 8 tubes each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

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