Venor®GeM Advance

Pre-aliquoted 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 extractions

Manufacturer

INDICATION

The *Venor®GeM Advance* is designed for direct detection of mycoplasma contamination in cell cultures.

TEST PRINCIPLE

Venor®GeM Advance utilizes the polymerase chain reaction (PCR), which is established as the method of choice for highly sensitive detection of *Mycoplasma* and *Acholeplasma* contamination in cell cultures and other cell culture derived biologicals. Detection requires as little as 5 fg of mycoplasma DNA corresponding to 20 mycoplasma per sample volume. The detection procedure can be performed within 3 hours. *Venor®GeM Advance* is intended for research use only, not for clinical diagnostics or testing of human samples.

The primer set is specific to the highly conserved 16S rRNA coding region in the mycoplasma genome. This allows the 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* and *Ureaplasma* species. Cross-detection of the following bacteria is not monitored: *Clostridium acetobutylicum*, *Lactobacillus acidophilus* and *Streptococcus pneumoniae*. Furthermore, human DNA is not detectable with this kit. See further details on page 6.

The provided reaction tubes are pre-coated with lyophilized Taq polymerase, nucleotides, primer, and loading dye to significantly reduce the hands-on time. Hence, the PCR product can be loaded directly on the agarose gel. *The pre-coated tube*s also contain an internal control DNA to verify the PCRs' success.

The reaction mixture contains dUTP instead of dTTP, so the option is available to degrade amplicons from previous analysis by use of uracil-DNA glycosylase (UNG). Thus, the occurrence of false-positive result can be minimized. UNG is not part of the product.

COMPONENTS

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 are stored until use at +2 to +8 °C and must be stored after opening and rehydration at < -18 °C.

Component	Description	Content
Test Reaction Tubes	tubes pre-coated with freeze-dried primers, polymerase, dNTPs, internal control DNA, and gel loading buffer/dye	3, 6, 12, or 30 strips of 8 transparent tubes each, depending on package size
Positive Control Reaction Tubes	Test Reaction Tubes containing additional DNA of *Mycoplasma orale* genome	1, 2, 3, or 5 strips of 8 red tubes each, depending on package size
Rehydration Buffer		1, 2, or 5 tubes à 1.6 ml, depending on package size
Caps for PCR Tubes		4, 6, 15, or 35 strips of 8 tubes each, depending on package size

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. Additional consumables and equipment is supplied by the user:

- PCR cycler
- Pipettes with corresponding filter tips (10 and 100 µl)
- 1.5 ml reaction tubes
- Agarose gel electrophoresis system including DNA stain

SPECIMEN FOR CELL CULTURE SCREENING

Samples should be obtained from cell cultures that are highly confluent (90 % or higher). 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, which may make is necessary to extract the DNA prior to the PCR test (see further information below). Note that penicillin or streptomycin in the culture media are not known to inhibit mycoplasma nor affect the tests' sensitivity.

The average mycoplasma number 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 successfully applying the PCR test. Prepare the PCR template as follows:

1.	Transfer 500 µl of the cell culture supernatant from the test culture to a 1.5 ml reaction tube. The lid should be sealed tightly to prevent opening during heating.
2.	Incubate the sample at 95 °C for 10 minutes.
3.	Briefly centrifuge (5 seconds) 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 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. 56-1050) 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 4 °C for up to 6 days at –20 °C for long time storage.

PRECAUTIONS

The *Venor®GeM Advance* 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 suitable lab coat and disposable gloves.

This kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

Cross contamination 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 Advance* kit. The components supplied should not be mixed with reagents from different lot and 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 can affect the results.

⇒ Avoid cross contamination by preparing the Positive Controls after the Negative Controls and Test Reactions

⇒ Set up at least one negative control sample (non template control) in each PCR. Use fresh cell culture medium or elution buffer for the NTC in case of extracted DNA.

⇒ PCR inhibition is likely to be caused by the sample matrix, or, in case of extracted DNA, caused by the elution buffer. Thus we recommend our *Venor®GeM Sample Preparation* kits. Any other DNA extraction kit needs to be qualified.

⇒ The appearance of the pellets in the pre-coated PCR tubes may vary on technical reasons which has no effect on the outcome of the test.

PROCEDURE

⇒ Set up negative and positive controls with each test.

⇒ The components must be equilibrated to room temperature prior use.

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 the Rehydration Buffer. **Note:** Rehydrate Positive Control Tubes with 25 µl of Rehydration Buffer.

2. Add samples

Follow the pipetting sequence and close tubes after the sample was added.

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 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 from 4 °C to 8 °C
3.	Start the program.

4. Agarose gel electrophoresis

1.	Cast a 1.5 or 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).
4.	Visualize the PCR results on a suitable transilluminator.

RESULT INTERPRETATION

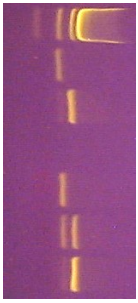
The *Internal Control DNA* gives rise to a distinct 191 bp band in every lane indicating a successfully performed PCR. This band will fade out with increased amounts of primary target amplification (e.g. mycoplasma DNA input of > 5 x 10^6 copies per ml. The initial concentration of positive control DNA is higher than 5 x 10^6 copies per ml. 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 (lower band intensity compared to negative control) a DNA extraction needs to be performed prior to re-testing the sample (see chapter "Specimen").

Detection of *Mycoplasma* band at 265-278 bp	Internal control band at 191 bp	Interpretation
Positive	Irrelevant	Mycoplasma present in the sample
Negative	Negative	PCR inhibition
Negative	Positive	No mycoplasma 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

ANALYTICAL CHARACTERISTICS OF THE TEST

Analytical Sensitivity

The detection limit was determined with 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.

Cross Reactivity

A cross-reactivity with eukaryotic DNA origin could not be found. Rarely unspecific PCR products can be formed and become visible in the gel as faint, diffuse bands of different sizes due to overloading the PCR (ref. chapter "Interpretation of Results"). The kit is also not detecting any of the phylogenetically related microorganisms, *Clostridium*, *Lactobacillus* and *Streptococcus*. Likewise, the water-born germ *Burgholderia* is not detected. But, at concentrations above 10^4 genomes/µl the test is positive for *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis.*

Sequence Alignment

A large number of *Mollicutes* sequences have been published. The primers of the kit were aligned with the NCBI data base and inspected for homologies within the target region of the 16S rRNA. At least 1 *Ureaplasma*, 7 *Acholeplasma* and 85 *Mycoplasma* show relevant sequence homologies and highest presumption of a positive PCR result.

Positive tested *Mollicutes*	Negative tested		
	EP listed bacteria	Other bacteria	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 genitalium		*Enterococcus faecalis*	Murine genomic DNA
Mycoplasma hominis		*Enterobacter aerogenes*	Calf thymus DNA
Mycoplasma hyorhinis		*Escherichia coli*	Foetal bovine serum
Mycoplasma orale		*Proteus mirabilis*	Horse serum
Mycoplasma penetrans		*Bacillus cereus*	Goat serum
Mycoplasma pneumoniae			
Mycoplasma salivarium			
Mycoplasma synoviae			
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.

Trademarks

Venor® is a registered trademark of Minerva Biolabs GmbH, Germany.

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
11-8025/-8050/-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/-1050/-1100/-1250	Onar® Bacteria Detection Kit	25/50/100/250 tests

Sample Preparation

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 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, 1 x 10^8 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/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 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 x 1000 ml
15-1001	Surface disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

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

WaterShield™

15-3025/-3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

Manufacturer

Minerva Biolabs GmbH
Koepenicker Str. 325
D-12555 Berlin
Germany

Ordering

Tel. +49 (0)30 2000 437-0
Fax +49 (0)30 2000 437-9
order@minerva-biolabs.com

Product Information

www.minerva-biolabs.com
info@minerva-biolabs.com

Technical Service

Tel. +49 (0)30 2000 437-40
support@minerva-biolabs.com

Made in Germany

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