10CFU™ Sensitivity Standards

For validating robustness and detection limit of molecular mycoplasma test methods in presence of the sample matrix.

Application

European Pharmacopoeia 2.6.7/*Japanese Pharmacopoeia, 17. edition, chapter G3* "Mycoplasma" requires a sensitivity of 10 CFU/ml sample volume for NAT-based methods like PCR to replace the traditional culture method. This feature of the test method must be shown by the performing lab as part of the robustness testing in presence of the sample matrix. As most cell culture labs and production facilities cannot accept vital mycoplasma in their facility or do not have access to a microbiology lab able to cultivate mycoplasma, these preparations allow safe and reliable validation of the procedure.

The mycoplasma have been cultivated in culture broth described in EP 2.6.7/ JP, 17. edition, chapter G3, titrated immediately in culture broth and plated for quantification in colony forming units (CFU/ml). Each dilution series has been performed in multiple by different operators for highest precision. The mycoplasma broth was harvested in the early logarithmic phase of the growth to avoid a high ratio of dead mycoplasma particles and correspondingly a high GU*:CFU ratio. All strains have been obtained from official culture collections and cultivated in low passages.

Each vial contains 10 CFU of inactivated mycoplasma. By adding the sample matrix of interest a sample according to EP 2.6.7/ JP, 17. edition, chapter G3 is prepared which has to be tested positive by the method applied. Obviously, the inactivated sample material is not suitable for the culture method anymore. As a result of proficiency tests on DNA amplification methods for mycoplasma detection it became obvious that in means of highest sensitivity DNA extraction is indispensible. The extract can directly be used for PCR.

* Please note: This standard material was not titrated for genome copies (GU) as EP 2.6.7/ JP, 17. edition, chapter G3 does not provide sensitivity limits on DNA level. No guarantee for a particular GU:CFU ratio is provided with this product and the ratio may vary from lot to lot.

Package Content

3 vials with 10 CFU of the corresponding mycoplasma species
2 negative control vials
For the mycoplasma set: 2 vials with 10 CFU of each mycoplasma species listed in the EP 2.6.7/ JP, 17. edition, chapter G3 (18 vials in total)
2 negative controls

Catalogue Number

102-1003	*Mycoplasma arginini*	102-7003	*Mycoplasma hyorhinis*
102-2003	*Mycoplasma orale*	102-8003	*Acholeplasma laidlawii*
102-3003	*Mycoplasma gallisepticum*	102-9003	*Spiroplasma citri*
102-4003	*Mycoplasma pneumoniae*	102-1103	*Mycoplasma salivarium*
102-5003	*Mycoplasma synoviae*	102-0002	Mycoplasma Set
102-6003	*Mycoplasma fermentans*		

PCR Quantification Standards

Application

- Performance controls for conventional and real-time PCR
- Standard curves for quantification

The PCR Quantification Standards contain genomic DNA which was extracted at low passage from defined microorganisms. The DNA is manufactured by means of phenol/chloroform extraction with ethanol precipitation and subsequent column absorption methods.

The DNA extract was partially sequenced and the sequence aligned to confirm identity. Titration was done after fluorometric quantification of the preparation against calibrated plasmid DNA. QC includes qPCR against a synthetic and highly defined control plasmid.

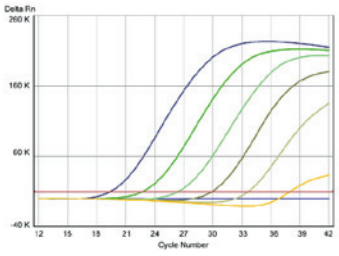
Package Content

1 vial with DNA, 1x10^8 genomes, freeze-dried, 3 vials with 2 ml of Tris-HCl buffer, 10 mM, pH 8.5., for dissolving the DNA and preparing dilutions

Order information

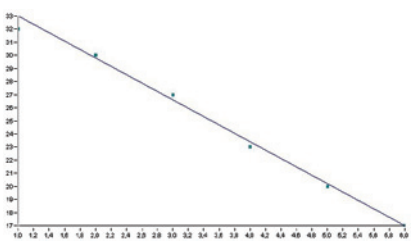
Cat. No. 52-0116	*Acholeplasma laidlawii*
Cat. No. 52-5571	*Bordetella pertussis*
Cat. No. 52-0083	*Escherichia coli*
Cat. No. 52-0101	*Legionella pneumophila*
Cat. No. 52-0129	*Mycoplasma* arginini
Cat. No. 52-0117	*Mycoplasma fermentans*
Cat. No. 52-0115	*Mycoplasma gallisepticum*
Cat. No. 52-0130	*Mycoplasma hyorhinis*
Cat. No. 52-0112	*Mycoplasma orale*
Cat. No. 52-0119	*Mycoplasma pneumoniae*
Cat. No. 52-0103	*Mycoplasma salivarium*
Cat. No. 52-0124	*Mycoplasma synoviae*
Cat. No. 52-0164	*Spiroplasma citri*
Cat. No. 52-0071	*Pseudomonas aeruginosa*

Real-time Amplification Plot



Cycle Number

Standard Curve



Log Concentration

Fig. Quantification of *Mycoplasma pneumoniae* DNA. Logarithmic plot of fluorescence vs cycle number (Venor®GeM qEP, platform: ABI Prism® 7500). Template DNA ranging from 2x10^5 - 2 genome equivalents.

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Mycoplasma Detection Kits

Minerva takes the advantage of long standing experiences and extended research in molecular testing. In combination with high quality manufacturing standards, this knowledge guarantees excellent test kits for the detection of mycoplasma contaminations in cell cultures and biopharmaceutical products.

Economic

- Select from 5 product variants with different validation levels and technical configurations according to your requirements
- PCR mix is provided in aliquots of 25 reactions for highest convenience and long term stability for occasional users.
- Critical kit components are provided freeze-dried for easy logistics, storage and best reagent stability.

Flexible

- Applicable for fast and reliable screening of cell cultures in research, EP-compliant lot release testing of ATMPs, in-process testing, raw material testing, etc.
- Kits are compatible with almost any commercially available PCR/qPCR device.

High Performance

- Highest robustness, sensitivity and specificity with a protocol easy to apply.
- Venor®GeM Classic and Venor®GeM qEP are validated comprehensively according to the European Pharmacopoeia 2.6.7/ Japanese Pharmacopoeia, 17. edition, chapter G3. The separate Internal Amplification Control allows for optional process monitoring.
- Superior results in proficiency tests and in depth robustness studies.

MB minerva biolabs®

Venor®GeM Classic

Description
Venor®GeM Classic is a basic PCR kit for fast, reliable and time-saving routine monitoring of mycoplasma contamination.

Recommended use / scope
Applicable in research and industry:
For direct screening of cell cultures and biologicals.
For EP 2.6.7/ JP, 17. edition, chapter G3 compliant release testing.

Type of PCR
Conventional, endpoint PCR

Device requirements
PCR cycler / Agarose gel electrophoresis and DNA staining system / Pipetting equipment / Tube centrifuge

Kit components
Primer & nucleotides / Internal amplification control DNA / Rehydration buffer / Positive control DNA / PCR grade water

Sample volume per PCR
2 µl for screening / 10 µl for EP 2.6.7/ JP, 17. edition, chapter G3 compliant testing

EP 2.6.7/ JP, 17. edition, chapter G3 compliance
Yes, after appropriate sample preparation and process validation

Validation
Validation report available on request

Result evaluation
Gel analysis

Required consumables
PCR reaction tubes / Polymerase / Gel loading buffer and dye

Optional consumables
For process monitoring:
Internal Control DNA extra (Cat. No. 11-1905)

Optional for process validation according to EP 2.6.7/JP, 17. edition, chapter G3:
10CFU™ Sensitivity Standards available for all EP-listed mycoplasma species (e.g. Mycoplasma orale, Cat. No. 102-2003)

Venor®GeM Sample Preparation Kit:
(Cat. No. 56-1050, 56-1200 or Cat. No. 56-2096)

Venor®GeM Advance

Description
Venor®GeM Advance contains PCR reaction tubes pre-coated with all PCR reagents including polymerase to reduce the total assay time without need to prepare aliquots of a master mix. For additional convenience the gel loading buffer and dye are already included in the reaction buffer. After thermal cycling the PCR can be loaded directly on the agarose gel.

Recommended use / scope
Applicable in research for direct testing of cell cultures and cell culture derived biologicals.

Type of PCR
Conventional, endpoint PCR

Device requirements
PCR cycler / Agarose gel electrophoresis and DNA staining system / Pipetting equipment / Tube centrifuge

Kit components
Primer sets, nucleotides, polymerase and internal amplification control DNA, filled in 0.2 ml PCR reaction tubes / Rehydration buffer including gel loading buffer and running dye / Positive controls, filled in 0.2 ml PCR reaction tubes / PCR grade water

Sample volume per PCR
2 µl

Validation
Not provided

EP 2.6.7/JP, 17. edition, chapter G3 compliance
No

Result evaluation
Gel analysis

Required consumables
None

Venor®GeM OneStep

Description
Venor®GeM OneStep is a complete kit which includes all reagents required for PCR. Primer, nucleotides, polymerase and the internal amplification control are provided ready-to-use in a freeze-dried reaction mix. The included rehydration buffer is added to the mix, aliquots made according to the sample number, sample or Positive Control DNA added and the setup is ready for PCR

Recommended use / scope
Applicable in research for direct testing of cell cultures and cell culture derived biologicals.

Type of PCR
Conventional, endpoint PCR

Device requirements
PCR cycler / Agarose gel electrophoresis and DNA staining system / Pipetting equipment / Tube centrifuge

Kit components
Primer sets, nucleotides, internal amplification control and polymerase / Rehydration buffer / Positive control DNA / PCR grade water

Sample volume per PCR
2 µl

Validation
Not provided

Required consumables
PCR reaction tubes / Gel loading buffer and dye

Result evaluation
Gel analysis

EP 2.6.7/JP, 17. edition, chapter G3 compliance
No

Venor®GeM qOneStep

Description
Venor®GeM qOneStep is a mycoplasma qPCR detection kit which includes all reagents required for the qPCR reaction. Primer, nucleotides, polymerase and the internal amplification control are provided ready-to-use in a lyophilized reaction mix. Rehydration buffer and lyophilized Positive Control DNA are also provided in the kit. The protocol provided is recommended for the fast and reliable screening of cell culture supernatants. Eukaryotic DNA is not amplified by this primer/probe system.

Recommended use / scope
Applicable in research for direct testing of cell cultures and cell culture derived biologicals. Not applicable for clinical diagnostics.

Type of PCR
TaqMan®-based quantitative real-time PCR

Device requirements
qPCR cycler with FAM™ and HEX™ filters / Pipetting equipment / Tube centrifuge

Kit components
Primer sets, nucleotides, internal amplification control and polymerase / Rehydration buffer / Positive control DNA / PCR grade water

Required consumables
PCR reaction tubes

Sample volume per PCR
2 µl

Validation
Not provided

Result evaluation
Cycler-based, real-time PCR

EP 2.6.7/JP, 17. edition, chapter G3
No

Venor®GeM qEP

Description
Venor®GeM qEP utilizes quantitative, real-time PCR for high quality and reliable detection of mycoplasma contamination. It can be used in combination with cell culture enrichment, for direct screening of cell cultures or after DNA extraction for EP compliant testing of cell culture derived biologicals, like autologous transplants (ATMPs), sera, cell culture media and therapeutic antibody formulations. Not applicable for clinical diagnostics.

Recommended use / scope
Applicable in research and industry:
For direct screening of cell cultures and biologicals.
For EP 2.6.7/JP, 17. edition, chapter G3 compliant release testing.

Type of PCR
Probe assay for qPCR

Device requirements
qPCR cycler with FAM™ and HEX™ filters / Pipetting equipment / Tube centrifuge

Kit components
Primer sets, nucleotides and polymerase / Rehydration buffer / Internal amplification control / Positive control DNA / PCR grade water

Sample volume per PCR
2 µl for screening / 10 µl for **EP 2.6.7/JP, 17. edition, chapter G3** compliant testing

EP 2.6.7/JP, 17. edition, chapter G3 compliance
Yes, after appropriate sample preparation and process validation

Validation
Validation report available on request

Result evaluation
Cycler based, real-time PCR

Required consumables
PCR reaction tubes

Optional consumables
For process monitoring and EP 2.6.7/JP, 17. edition, chapter G3 testing:
Internal Control DNA extra (Cat. No. 11-9905)

Venor®GeM Sample Preparation Kit:
(Cat. No. 56-1050, 56-1200 or Cat. No. 56-2096)

For process validation according to EP 2.6.7/JP, 17. edition, chapter G3:
10CFU™ Sensitivity Standards available for all EP-listed mycoplasma species (e.g. Mycoplasma orale, Cat. No. 102-2003)

Storage (applies to all kits)
Components can be stored at +2 to +8°C for at least 12 months. After rehydration the reagents must be stored at ≤ -18°C.

Ordering information / package sizes

Venor®GeM Classic	Venor®GeM Advance	Venor®GeM OneStep	Venor®GeM qOneStep	Venor®GeM qEP
Cat. No. 11-1025 25 Tests	Cat. No. 11-7024 24 Tests	Cat. No. 11-8025 25 Tests	Cat. No. 11-91025 25 Tests	Cat. No. 11-9025 25 Tests
Cat. No. 11-1050 50 Tests	Cat. No. 11-7048 48 Tests	Cat. No. 11-8050 50 Tests	Cat. No. 11-91100 100 Tests	Cat. No. 11-9100 100 Tests
Cat. No. 11-1100 100 Tests	Cat. No. 11-7096 96 Tests	Cat. No. 11-8100 100 Tests	Cat. No. 11-91250 250 Tests	Cat. No. 11-9250 250 Tests
Cat. No. 11-1250 250 Tests	Cat. No. 11-7240 240 Tests	Cat. No. 11-8250 250 Tests		