

# Venor® GeM qEP

Mycoplasma Detection Kit for qPCR

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## INSTRUCTIONS FOR USE

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**FOR USE IN RESEARCH AND QUALITY CONTROL**

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## Symbols

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**Lot No.**



**Cat. No.**



**Expiry date**



**Storage temperature**



**Number of reactions**



**Manufacturer**

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## INDICATION

The Venor®GeM qEP Kit is designed for the quantitative detection of *Mollicutes*, such as *Mycoplasma* (frequently used interchangeably with *Mollicutes*), *Acholeplasma*, and *Spiroplasma*, in cell cultures and other biological matrices.

The Venor®GeM qEP Kit is based on real-time or quantitative PCR (qPCR), as the established method of choice for rapid, robust and sensitive detection of *Mollicutes*. The assay is targeting a highly conserved region within the mycoplasma genome to detect all species included in the European Pharmacopoeia Chapter 2.6.7 (EP 2.6.7), in the Japanese Pharmacopoeia Chapter G3 (JP G3) and many more (see list in the section “Assay Characteristics”). The product has been successfully tested with a broad range of qPCR devices.

The kit design meets the test criteria of the EP 2.6.7 and JP G3 with different kinds of sample material (chondrocytes, serum, cell culture supernatant, etc.) after DNA extraction of the samples. The assay is suitable for the direct detection in cell culture supernatants usually applicable in research, or for performing the “cell culture enrichment” method, or after DNA extraction. The kit fully complies with the requirements of EP 2.6.7 and JP G3.

## TEST PRINCIPLE

*Mollicutes* (see list in the section “Assay Characteristics”) are specifically detected by amplifying the 16S rRNA coding region, whereas eukaryotic and other bacterial DNA are not amplified by the Venor®GeM qEP Kit.

The user instructions include protocols for both screening of cell culture supernatants as well as EP- and JP-compliant testing with defined DNA extraction and sample volume specifications. 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 approaches.

The kit contains all necessary PCR components including hot-start Taq polymerase, primers, and dNTPs in a lyophilized mix (Mycoplasma Mix) as well as Rehydration Buffer and PCR Grade Water. False-negative results caused by PCR inhibition and/or DNA extraction issues will be reliably identified by means of the Internal Control DNA. The Internal Control DNA is either added directly to the PCR master mix to function as a PCR performance control, or alternatively, added to the original sample prior to DNA extraction. In the latter case, both the DNA extraction and the qPCR amplification are validated. Additional Internal Control DNA can be purchased from Minerva Biolabs (Cat. No. 11-9905). The amplification of the Internal Control DNA is detected at 560 nm (HEX™ channel), whereas the mycoplasma-specific amplification is detected at 520 nm (FAM™ channel).

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 qEP Kit.

## CONTENT

Each kit contains reagents for 25, 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 components must be stored at ≤ -18 °C.

| Component            | Quantity                            |                                      |                                      | Cap color |
|----------------------|-------------------------------------|--------------------------------------|--------------------------------------|-----------|
|                      | 25 reactions<br>Cat. No.<br>11-9025 | 100 reactions<br>Cat. No.<br>11-9100 | 250 reactions<br>Cat. No.<br>11-9250 |           |
| Mycoplasma Mix       | 1 vial<br>lyophilized               | 4 vials<br>lyophilized               | 10 vials<br>lyophilized              | red       |
| Rehydration Buffer   | 1 vial<br>1.8 ml                    | 1 vial<br>1.8 ml                     | 3 vial<br>1.8 ml each                | blue      |
| Positive Control DNA | 1 vial<br>lyophilized               | 1 vial<br>lyophilized                | 2 vials<br>lyophilized               | green     |
| Internal Control DNA | 1 vial<br>lyophilized               | 2 vials<br>lyophilized               | 4 vials<br>lyophilized               | yellow    |
| PCR Grade Water      | 1 vial<br>2 ml                      | 1 vial<br>2 ml                       | 1 vial<br>2 ml                       | white     |

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website ([www.minerva-biolabs.com](http://www.minerva-biolabs.com) / [www.minervabiolabs.us](http://www.minervabiolabs.us)).

## USER-SUPPLIED CONSUMABLES AND EQUIPMENT

Venor®GeM qEP Kit contains reagents for the specific detection of *Mollicutes*. Additional consumables and equipment are supplied by the user:

- qPCR device with filter sets for detecting the fluorescent dyes FAM™ and HEX™
- PCR reaction tubes or plates and caps, recommended by the manufacturer of your thermal cycler
- Microcentrifuge for 1.5 ml reaction tubes
- Pipettes with corresponding filter tips (10, 100, and 1000 µl)
- Required for EP 2.6.7/JP G3 compliant testing:  
DNA extraction kit, e.g. Venor®GeM Sample Preparation Kit (Cat. No. 56-1050)
- Required for cell culture screening (only with Cat. No. 11-9250): PCR Grade Water
- Optional for EP 2.6.7/JP G3 compliant testing:  
10 mM Tris-HCl buffer, pH 8.4
- Optional for process validation and EP 2.6.7/JP G3 compliant testing:  
Internal Control DNA extra (Cat. No. 11-9905)  
10CFU™ Sensitivity Standards available for all EP-/JP-listed mycoplasma species (see „Related Products“)  
Proteinase K (Cat. No. 56-0002)
- Optional for carry-over prevention: Uracil DNA glycosylase (UNG)

## SAMPLES FOR CELL CULTURE SCREENING

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 cultures medium, which will make 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 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 to 500  $\mu$ l of the supernatant from the cell culture to a 1.5 ml reaction tube. Close the lid tightly.
- 
2. Incubate the sample at 95 °C for 10 min (at least 5 min).
- 
3. Centrifuge the sample for 30 sec at max. speed (e.g. 10,000  $\times$  g) to pellet cellular debris.
- 
4. Use 2  $\mu$ l of the supernatant directly for qPCR, or store the sample for up to 6 days at +2 to +8 °C or at  $\leq -18$  °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 ( $> 2 \mu$ l), 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<sup>®</sup>GeM qEP Kit was tested in combination with Venor<sup>®</sup>GeM Sample Preparation Kit (Cat. No. 56-1010/-1050/-1200) for mycoplasma DNA extraction.

Extracted DNA may be stored at +2 to +8 °C for up to 6 days. Long-term storage must be at  $\leq -18$  °C.

## SAMPLES FOR EP 2.6.7 / JP G3 COMPLIANT TESTING

### 1. Sample concentration (optional)

The sample matrix might have a critical influence on the sensitivity of the detection method. An approach to further enhance the sensitivity of the assay is to collect and screen higher sample volumes of cell culture supernatants than those required for mycoplasma DNA extraction (e.g. > 200  $\mu$ l, for our Venor®GeM Sample Preparation Kit) and perform a sample concentration step.

Please note that the sample concentration protocol works only with intact mycoplasma cells. Therefore, any procedure disrupting the cells (e.g. by heat inactivation, see „2. Sample stabilization“) prior to sample concentration must be avoided. Samples up to 200  $\mu$ l volume can be processed directly without a concentration step.

- 
1. Transfer up to 1 ml supernatant of the sample into a 1.5 ml reaction tube.
- 
2. Centrifuge the sample at  $\geq 10,000 \times g$  for 15 min (or  $\geq 13,000 \times g$  for 6 min) to pellet mycoplasma particles.
- 
3. Discard the supernatant and resuspend the pellet in 200  $\mu$ l Tris buffer (10 mM Tris-HCl, pH 8.4).
- 
4. Vortex the sample briefly and proceed immediately with sample stabilization (2.2.) or DNA extraction.
- 

### 2. Sample stabilization (optional)

Cell culture samples are likely to contain high concentrations of DNases which will degrade mycoplasma DNA even at lower temperatures. Therefore, we recommend the following steps to stabilize the sample. This step is not necessary if DNA extraction is performed immediately after sample collection. Please note that sample stabilization by heat inactivation cannot be performed before the sample concentration step.

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Transfer 500  $\mu$ l of the supernatant from the cell culture into a 1.5 ml reaction tube.

1. Close the lid tightly.  
Note: Skip this step when performing the sample concentration procedure
- 
2. Incubate the sample at 95 °C for 10 min (at least 5 min).
- 
3. Centrifuge the sample for 30 sec at max. speed (e.g. 10,000  $\times g$ ) to pellet cellular debris.
- 
4. Use the sample for DNA extraction. Store the sample for up to 6 days at +2 to +8 °C or at  $\leq -18$  °C for long-term storage.
- 

### 3. DNA extraction (required)

A substantial body of evidence shows that DNA extraction is required to achieve the highest level of sensitivity. Numerous DNA extraction methods are established for a vast variety of sample materials. However, the DNA extraction method must be compatible for mycoplasma genomes. For EP-/JP compliant testing, the DNA extraction method must be tested in combination with the PCR kit. We recommend our Venor®GeM Sample Preparation Kit (Cat. No. 56-1010/-1050/-1200). This DNA extraction kit was tested extensively. Please see the Instructions for Use of the Venor®GeM Sample Preparation Kit for the detailed protocol.

## RECOMMENDATIONS

Ideally, the Internal Control DNA of the Venor®GeM qEP Kit is used to verify the DNA extraction step as well.

Please note, that the actual sample volume that will be spiked is not relevant for the required volume of Internal Control DNA. The volume of Internal Control DNA depends on the final elution volume (containing the DNA extract) of the DNA extraction step. In general, add 2  $\mu\text{l}$  per 10  $\mu\text{l}$  DNA extract to the sample, vortex briefly and proceed with the DNA extraction. (Example: add 12  $\mu\text{l}$  Internal Control DNA to the original sample if the elution volume will be 60  $\mu\text{l}$  Elution Buffer.)

Do not add further Internal Control DNA to the qPCR master mix if the internal control was already added to the sample before.

Note that this is not compatible with the sample concentration step as the sample concentration requires intact cells. Thus add the Internal Control DNA either prior to the sample stabilization or DNA extraction step.

Internal Control DNA can be purchased separately (Internal Control DNA extra, Cat. No. 11-9905).

## PRECAUTIONS

The Venor®GeM qEP Kit is for *in vitro* 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.

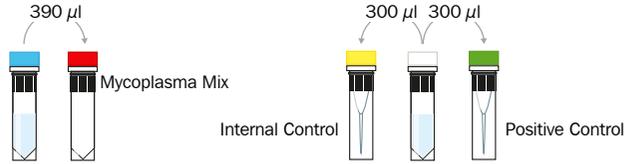
## ADDITIONAL NOTES

- ⇒ These instructions must be understood to successfully use the Venor®GeM qEP Kit. The reagents supplied should not be mixed with reagents from different batches 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 the 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 for sample preparation. 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.
- ⇒ In order to minimize the number of freeze-thawing cycles, rehydrate the Mycoplasma 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

-  Mycoplasma Mix
-  Internal Control
-  Positive Control

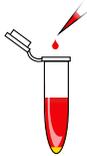


-  5 min RT
-  briefly
-  for 5 sec

## 2. Reaction mix preparation

for EP/JP compliant testing

- 1 reaction**  
 15 µl Mycoplasma Mix  
 1 µl Internal Control



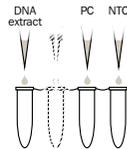
for cell culture screening

- 1 reaction**  
 15 µl Mycoplasma Mix  
 8 µl PCR grade water  
 1 µl Internal Control

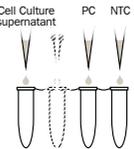


## 3. Add samples

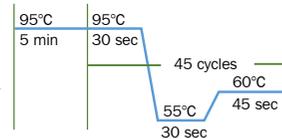
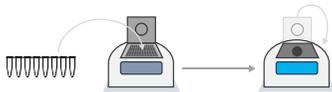
- 15 µl reaction mix  
 + 10 µl of DNA extract  
 or  
 + 10 µl Positive Control (PC)  
 or  
 + 10 µl Elution Buffer (NTC)



- 23 µl reaction mix  
 + 2 µl cell culture supernatant  
 or  
 + 2 µl Positive Control (PC)  
 or  
 + 2 µl PCR grade water (NTC)



## 4. Start PCR amplification



-  Rehydration Buffer
-  Mycoplasma Mix
-  PCR grade water

-  Positive Control
-  Internal Control

-  add
-  incubate
-  vortex

-  centrifuge
- NTC = No Template Control
- PC = Positive Control

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

MB\_SI\_VGM-qEP\_05\_EN

## PROCEDURE - STEP BY STEP

### 1. Reagent preparation

The test should be carried out with negative and positive controls and samples in duplicates. After reconstitution, the reagents should be stored at  $\leq -18\text{ }^{\circ}\text{C}$ . Repeated freezing and thawing should be avoided and reconstituted controls (internal control and positive control) stored in aliquots.

|    |  |                                    |   |
|----|--|------------------------------------|---|
| 1. | Mycoplasma Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>Green cap | Spin down all lyophilized components at max speed for 5 sec.  |
| 2. | Mycoplasma Mix   | Red cap                            | Add 390 $\mu\text{l}$ Rehydration Buffer (blue cap)<br>(For sample kits (10 reactions): add 160 $\mu\text{l}$ ) |
| 3. | Internal Control DNA   | Yellow cap                         | Add 300 $\mu\text{l}$ PCR Grade Water (white cap)   |
| 4. | Positive Control DNA   | Green cap                          | Add 300 $\mu\text{l}$ PCR Grade Water (white cap)   |
| 5. | Mycoplasma Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>Green cap | Incubate at room temperature for 5 min  |
| 6. | Mycoplasma Mix<br>Internal Control DNA<br>Positive Control DNA | Red cap<br>Yellow cap<br>Green cap | Vortex and spin down for 5 sec  |

### 2. Reaction mix preparation

Follow these schemes and sequences to set up the test:

Prepare the required volume of master mix for the number of samples. Mix the kit components at room temperature in a 1.5 ml reaction tube for all control and test reactions.

| <b>EP-/JP-compliant testing:</b> |   | Cap Colour             | for 1 reaction   | for 25 reactions   |
|----------------------------------|---|------------------------|--|--|
| 1.                               | Mycoplasma Mix<br>Internal Control DNA  | Red<br>Yellow          | 15 $\mu\text{l}$<br>1 $\mu\text{l}$                    | 375 $\mu\text{l}$<br>25 $\mu\text{l}$                      |
| <b>Cell culture screening:</b>   |   | Cap Colour             | for 1 reaction   | for 25 reactions   |
|                                  | Mycoplasma Mix<br>PCR Grade Water<br>Internal Control DNA   | Red<br>White<br>Yellow | 15 $\mu\text{l}$<br>8 $\mu\text{l}$<br>1 $\mu\text{l}$ | 375 $\mu\text{l}$<br>200 $\mu\text{l}$<br>25 $\mu\text{l}$ |
| 2.                               | Homogenize the master mix by pipetting (5-times).   |                        |  |  |
| 3.                               | Pipet either 15 $\mu\text{l}$ (for EP- / JP-compliant testing) or 23 $\mu\text{l}$ (for cell culture screening) of the master mix to each PCR tube, discard remaining material. |                        |  |  |

### 3. Add samples

⇒ Set up negative (no template controls, NTCs) and positive controls in each PCR.

Prepare NTCs:

1. for EP-/JP-compliant testing: add 10  $\mu$ l elution buffer supplied with the DNA extraction kit  
for cell culture screening: add 2  $\mu$ l water or fresh cell culture medium or elution buffer

Add test samples:

2. for EP-/JP-compliant testing: add 10  $\mu$ l of DNA extract  
for cell culture screening: add 2  $\mu$ l of sample

Prepare Positive Control:

3. for EP-/JP-compliant testing: add 10  $\mu$ l of rehydrated Positive Control DNA  
for cell culture screening: add 2  $\mu$ l of rehydrated Positive Control DNA

4. Spin PCR tubes briefly and ensure that all tubes are closed tightly.

### 4. Start PCR amplification

1. Place PCR tubes in the qPCR device and close the lid.

Program the qPCR cycler or check stored temperature profiles.

Note: Visit our website for the Technical Note with detailed cycler programs of selected qPCR cyclers.

Programs for additional cyclers are available upon request.

2. 1 cycle 95 °C for 5 min  
45 cycles 95 °C for 30 sec  
55 °C for 30 sec  
60 °C for 45 sec  
Hold between +4 °C to +10 °C

3. Start the program.

This assay was tested on the following qPCR devices. A Technical Note with detailed cycler programs for selected qPCR cyclers is available on our website. We also recommend reviewing the cycling conditions and thermal profiles provided as templates by the manufacturer of your cycler.

| qPCR device                          | Manufacturer         |
|--------------------------------------|----------------------|
| CFX96™                               | Bio-Rad Laboratories |
| LightCycler® 2.0<br>LightCycler® 480 | Roche Diagnostics    |
| ABI Prism® 7500                      | Applied Biosystems   |
| Rotor-Gene® 6000                     | Corbett Research     |
| Mx3005P®                             | Agilent Technologies |
| AriaMx                               | Agilent Technologies |
| ABI StepOne™<br>StepOnePlus™         | Applied Biosystems   |

## DATA INTERPRETATION

The presence of *Mollicutes* is indicated by an increasing fluorescence signal in the FAM™ channel. The quantification is based on threshold cycle (Ct) values and a DNA standard curve. The exact procedure for obtaining Ct-values including baseline calculation/normalization depends on the particular qPCR device and cycler control software. Please see the documentation of your device for further details. We recommend the assessment of the amplification curve progression of all samples including control samples.

A positive PCR is indicated by  $C_t < 40$ . PCR reactions with  $C_t \geq 40$  are considered negative. In addition, a successful PCR is displayed by an increasing fluorescence signal in either the FAM™ or the HEX™ channel (given the Internal Control was added), or both. The mycoplasma DNA and Internal Control function as competitors in the PCR. Thus, the more mycoplasma DNA is in the sample, the higher the signal in the FAM™ channel and the lower the internal control signal in the HEX™ channel. The following table will help with the interpretation of PCR results:

| <b>Detection of <i>Mollicutes</i><br/>FAM™ channel</b> | <b>Internal control<br/>HEX™ channel</b> | <b>Interpretation</b>          |
|--|--|--------------------------------|
| positive   | irrelevant                               | Positive for <i>Mollicutes</i> |
| negative   | negative                                 | PCR inhibition                 |
| negative   | positive                                 | Negative for <i>Mollicutes</i> |

## ASSAY CHARACTERISTICS

### Sensitivity

The detection limit is determined by using the EP- / JP-compliant protocol with 10  $\mu$ l sample volume per PCR reaction. The required detection limit (10 CFU/ml) is reached on samples of cell culture medium (DMEM supplemented with 10 % FCS) spiked with 10 CFU/ml of all EP / JP listed mycoplasma species, after DNA extraction with Venor<sup>®</sup>GeM Sample Preparation Kit.

| Species                       | Detection limit LOD <sub>95</sub><br>[CFU/ml] | Species                         | Detection limit LOD <sub>95</sub><br>[CFU/ml] |
|-------------------------------|---|---------------------------------|---|
| <i>Acholeplasma laidlawii</i> | ≤ 10 CFU/ml                                   | <i>Mycoplasma pneumoniae</i>    | ≤ 10 CFU/ml                                   |
| <i>Mycoplasma hyorhinis</i>   | ≤ 10 CFU/ml                                   | <i>Mycoplasma arginini</i>      | ≤ 10 CFU/ml                                   |
| <i>Mycoplasma fermentans</i>  | ≤ 10 CFU/ml                                   | <i>Mycoplasma gallisepticum</i> | ≤ 10 CFU/ml                                   |
| <i>Mycoplasma orale</i>       | ≤ 10 CFU/ml                                   | <i>Spiroplasma citri</i>        | ≤ 10 CFU/ml                                   |
| <i>Mycoplasma synoviae</i>    | ≤ 10 CFU/ml                                   | <i>Mycoplasma salivarium</i>    | ≤ 10 CFU/ml                                   |

### Specificity

The qPCR assay detects further *Mollicutes* species (see below), but not any of the phylogenetically related microorganisms, such as *Clostridium*, *Lactobacillus*, and *Streptococcus*. Lack of cross-reactivity with other bacterial (a full list of tested microorganisms is provided in our validation study) and mammalian DNA is shown below. The test is positive for *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis* at concentrations above 0.1 ng/PCR, 1 ng/PCR, 0.05 ng/PCR, respectively.

| Positively tested<br><i>Mollicutes</i> | Negatively tested                 |                                       |                     |
|--|-----------------------------------|---------------------------------------|---------------------|
|  | EP/JP listed bacteria             | Other microorganisms                  | Mammals             |
| <i>Acholeplasma laidlawii</i>          | <i>Clostridium acetobutylicum</i> | <i>Acinetobacter baumannii</i>        | Vero-B4             |
| <i>Mycoplasma arginini</i>             | <i>Lactobacillus acidophilus</i>  | <i>Bordetella pertussis</i>           | CHO-K1              |
| <i>Mycoplasma arthritidis</i>          | <i>Streptococcus pneumoniae</i>   | <i>Campylobacter jejuni</i>           | RK13                |
| <i>Mycoplasma fermentans</i>           |                                   | <i>Clostridium perfringens</i>        | Human placenta      |
| <i>Mycoplasma gallisepticum</i>        |                                   | <i>Enterococcus casseliflavus</i>     | Foetal bovine serum |
| <i>Mycoplasma genitalium</i>           |                                   | <i>Geobacillus stearothermophilus</i> | Horse serum         |
| <i>Mycoplasma hominis</i>              |                                   | <i>Pseudomonas aeruginosa</i>         |                     |
| <i>Mycoplasma hyorhinis</i>            |                                   | <i>Salmonella enterica</i>            |                     |
| <i>Mycoplasma orale</i>                |                                   | <i>Staphylococcus aureus</i>          |                     |
| <i>Mycoplasma penetrans</i>            |                                   | <i>Staphylococcus saprophyticus</i>   |                     |
| <i>Mycoplasma pneumoniae</i>           |                                   | <i>Streptococcus dysgalactiae</i>     |                     |
| <i>Mycoplasma salivarium</i>           |                                   | <i>Streptococcus mutans</i>           |                     |
| <i>Mycoplasma synoviae</i>             |                                   | <i>Streptococcus sanguinis</i>        |                     |
| <i>Spiroplasma citri</i>               |                                   |                                       |                     |
| <i>Ureaplasma urealyticum</i>          |                                   |                                       |                     |

## 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*

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registered trademark of Applied Biosystems Corporation or its subsidiaries in the US and certain other countries. CFX96 Touch is a trademark of Bio-Rad Laboratories, Inc. Rotor-Gene is a registered trademark of the Qiagen Group. Mx3005P is a registered trademark of Stratagene, an Agilent Technologies company. StepOne and StepOnePlus are trademarks of Applied Biosystems or its subsidiaries in the U.S. and/or certain other countries. FAM and HEX are trademarks of Applied Biosystems or its subsidiaries. Venor, Mynox, Onar, and ZellShield are registered trademarks and PCR Clean, Mycoplasma Off, 10CFU, 100CFU, and WaterShield are trademarks of Minerva Biolabs GmbH, Germany.

## Related Products

### MB Taq DNA Polymerase

|                           |                                      |                      |
|---------------------------|--------------------------------------|----------------------|
| 53-0050/-0100/-0200/-0250 | MB Taq DNA Polymerase (5 U/ $\mu$ l) | 50/100/200/250 units |
| 53-1050/-1100/-1200/-1250 | MB Taq DNA Polymerase (1 U/ $\mu$ l) | 50/100/200/250 units |

### Contamination Control Kits for conventional PCR

|                           |   |                         |
|---------------------------|---|-------------------------|
| 11-1025/-1050/-1100/-1250 | Venor <sup>®</sup> GeM Classic Mycoplasma Detection Kit | 25/50/100/250 reactions |
| 11-7024/-7048/-7096/-7240 | Venor <sup>®</sup> GeM Advance Mycoplasma Detection Kit | 24/48/96/240 reactions  |
| 11-8025/-8050/-8100/-8250 | Venor <sup>®</sup> GeM OneStep Mycoplasma Detection Kit | 25/50/100/250 reactions |
| 12-1025/-1050/-1100/-1250 | Onar <sup>®</sup> Bacteria Detection Kit                | 25/50/100/250 reactions |

### Contamination Control Kits for qPCR

|                        |  |                      |
|------------------------|--|----------------------|
| 11-91025/-91100/-91250 | Venor <sup>®</sup> GeM qOneStep Mycoplasma Detection Kit | 25/100/250 reactions |
|------------------------|--|----------------------|

### Sample Preparation

|                     |   |                       |
|---------------------|---|-----------------------|
| 56-1010/-1050/-1200 | Venor <sup>®</sup> GeM Sample Preparation Kit | 10/50/200 extractions |
|---------------------|---|-----------------------|

### Mycoplasma Elimination

|                     |  |                   |
|---------------------|--|-------------------|
| 10-0200/-0500/-1000 | Mynox <sup>®</sup> Mycoplasma Elimination Reagent      | 2/5/10 treatments |
| 10-0201/-0501/-1001 | Mynox <sup>®</sup> Gold Mycoplasma Elimination Reagent | 2/5/10 treatments |

### 100CFU™ Sensitivity Standards, 3 vials with 100 CFU each, 2 vials negative control

|          |                                 |
|----------|---------------------------------|
| 103-1003 | <i>Mycoplasma arginini</i>      |
| 103-2003 | <i>Mycoplasma orale</i>         |
| 103-3003 | <i>Mycoplasma gallisepticum</i> |
| 103-4003 | <i>Mycoplasma pneumoniae</i>    |
| 103-1103 | <i>Mycoplasma salivarium</i>    |
| 103-5003 | <i>Mycoplasma synoviae</i>      |
| 103-6003 | <i>Mycoplasma fermentans</i>    |
| 103-7003 | <i>Mycoplasma hyorhinis</i>     |
| 103-8003 | <i>Acholeplasma laidlawii</i>   |
| 103-9003 | <i>Spiroplasma citri</i>        |

### 10CFU™ Sensitivity Standards, 3 vials with 10 CFU each, 2 vials negative control

|          |   |
|----------|---|
| 102-1003 | <i>Mycoplasma arginini</i>  |
| 102-2003 | <i>Mycoplasma orale</i>   |
| 102-3003 | <i>Mycoplasma gallisepticum</i>   |
| 102-4003 | <i>Mycoplasma pneumoniae</i>  |
| 102-1103 | <i>Mycoplasma salivarium</i>  |
| 102-1103 | <i>Mycoplasma synoviae</i>  |
| 102-6003 | <i>Mycoplasma fermentans</i>  |
| 102-7003 | <i>Mycoplasma hyorhinis</i>   |
| 102-8003 | <i>Acholeplasma laidlawii</i>   |
| 102-9003 | <i>Spiroplasma citri</i>  |
| 102-0002 | Mycoplasma Set, all EP 2.6.7 listed species, 2 vials per species, 10 CFU each |

### 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/ 3×50 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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HB11.08EN