

10CFU™ Sensitivity Standard

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols



Lot No.



Order No.



Expiry date



Storage temperature



Number of reactions



Manufacturer

INDICATION

10CFU™ sensitivity standards are intended for validating the robustness and sensitivity of NAT-based mycoplasma tests.

EXPLANATION OF THE TEST

Mycoplasma tests according to the *European Pharmacopoeia* (EP) Chapter 2.6.7 require a sensitivity of 10 colony forming units (CFU) per ml sample volume for NAT-based methods such as PCR in order to replace traditional culture methods. This sensitivity must be accomplished as part of the robustness testing for any particular sample matrix.

vital mycoplasma is not acceptable for the majority of cell culture laboratories due to safety regulations. Our 10CFU™ sensitivity standards contain non-vital material and allow safe and reliable validation.

The mycoplasma were grown in culture medium as described in EP 2.6.7, subsequently titrated and plated on Hayflick and Frey medium for CFU determination. The mycoplasma were harvested in the early logarithmic growth phase to ensure a high ratio of vital to non-vital mycoplasma and thus a low ratio of GU* to CFU. All strains were obtained from the National Collection of Type Cultures (NCTC), UK, and cultivated in low passages. The corresponding NCTC and ATCC codes are listed in the reagents table.

*GU = genome units

TEST PRINCIPLE

Each vial contains 10 CFU of irreversibly inactivated mycoplasma. Thus the mycoplasma cannot be used for culture methods. Briefly, a sample according to EP 2.6.7 is prepared by adding the sample matrix of interest.

The sample then needs to be subjected to DNA extraction procedure prior to PCR amplification. The DNA extraction is necessary as shown in numerous round robin test in order to achieve highest sensitivity. DNA extraction can be easily done using our Venor® GeM Sample Preparation Kit. The extracted DNA shall be used directly for PCR. The particular PCR test must show positive amplification for EP compliance.

Please note: Our 10CFU™ sensitivity standards were adjusted with respect to colony forming units and not for genome copies, as EP 2.6.7 does not include specifications on DNA levels. We do not provide specifications of GU:CFU ratio for our sensitivity standards. The GU:CFU ratio may vary between different lot.

REAGENTS TABLE

Kit component	Quantity	NCTC codes	ATCC codes	Cat. No.
<i>Mycoplasma arginini</i>		10129	23838	102-1003
<i>Mycoplasma orale</i>		10112	23714	102-2003
<i>Mycoplasma gallisepticum</i>		10115	19610	102-3003
<i>Mycoplasma pneumoniae</i>		10119	15531	102-4003
<i>Mycoplasma synoviae</i>	3 vials	10124	25204	102-5003
<i>Mycoplasma fermentans</i>		10117	19989	102-6003
<i>Mycoplasma hyorhinis</i>		10130	17981	102-7003
<i>Acholeplasma laidlawii</i>		10116	23206	102-8003
<i>Spiroplasma citri</i>		10164	27556	102-9003
Negative control	2 vials	n.a.	n.a.	n.a.

The 10CFU™ mycoplasma set (Cat. No. 102-0003) contains 2 vials of each *mollicutes* species listed in EP 2.6.7 (18 vials in total) as well as 2 vials of the negative control.

The mycoplasma were titrated directly in culture medium to ensure cell integrity and subsequently diluted ten-fold in 10 mM Tris buffer, pH 8.0, to avoid adverse culture medium effects on the PCR test. All samples were lyophilised for sustained product stability.

The mycoplasma were inactivated prior to lyophilisation. Lack of vitality was proven by media fills with Hayflick and Frey medium and cultivated at 37 °C (30 °C for *Spiroplasma citri*) for 23 days. Growth was monitored by quantitative PCR using the Venor®GeM qEP Mycoplasma Detection kit.

The date of expiry of the unopened product is given on the package label. The kit components must be stored until use at +2 to +8°C. The LOT-specific *Certificate of Analysis* can be downloaded from our website: www.minerva-biolabs.com.

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The kit contains reagents for the preparation of samples spiked with mycoplasma particles. Any other general laboratory equipment and consumables such as PCR reagents is supplied by the user.

PRECAUTIONS

The 10CFU™ sensitivity standards are for *in vitro* use only and should be applied by experienced laboratory staff. The kit does not contain hazardous substances. Remnants can be discarded according to local regulations.

TEST PROCEDURE

All reagents and samples must be equilibrated to room temperature before you start.

1. Spin the vials briefly to collect the lyophilised material at the bottom of the tube.

2. Add 1 ml of your sample material to each vial.

3. Incubate 5 min at room temperature.

4. Vortex for 10 sec and spin for 5 sec.

5. Use the appropriate volume for DNA extraction. We do recommend our Venor[®]GeM Sample Preparation Kit.

6. Use an appropriate volume of extracted DNA for PCR amplification.

The negative control samples were prepared as the sensitivity standards but do not contain any mycoplasma particles. The negative control must be rehydrated with the same sample matrix and processed in parallel with an appropriate number of replicates to verify the test results properly.

ADDITIONAL NOTES

The user instructions must be well understood for a successful application of this product. The reagents supplied must not be mixed with reagents from different lots but used as an integral unit only. The reagents must not be used beyond shelf life. Any deviation from the test protocol may compromise the results.

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.

Related Products

Contamination Control Kits

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
11-9025/-9100/-9250	Venor® GeM qEP Mycoplasma Detection Kit	25/100/250 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, 1x10⁸ genomes / vial

52-0112	<i>Mycoplasma orale</i>
52-0115	<i>Mycoplasma gallisepticum</i>
52-0116	<i>Acholeplasma laidlawii</i>
52-0117	<i>Mycoplasma fermentans</i>
52-0119	<i>Mycoplasma pneumonia</i>
52-0124	<i>Mycoplasma synoviae</i>
52-0129	<i>Mycoplasma arginini</i>
52-0130	<i>Mycoplasma hyorhinis</i>
52-0164	<i>Spiroplasma citri</i>

See Minerva homepage for further available species

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>	
102-5003	<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

DNA Remover™

15-2025/15-2200	DNA decontamination reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in 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
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WaterShield™

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