100CFU™ Sensitivity Standard

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

100CFU™ Sensitivity Standards are designed as complementary products to the 10CFU™ Sensitivity Standards (Cat. No. 102-XX03) to perform the validation of nucleic acid amplification technology (NAT)-based tests for mycoplasma detection.

The European Pharmacopoeia Chapter 2.6.7 (EP 2.6.7) and the Japanese Pharmacopoeia (17th Edition) Chapter G3 (JP G3) require the detection of 10 Colony Forming Units per ml of sample volume (CFU/ml), if NAT-based tests (e.g. PCR) are proposed as alternative methods to traditional culture assays. Such sensitivity must be demonstrated as part of the robustness testing for each specific sample matrix of interest. However, culturing or handling viable mycoplasma as reference tools is inadmissible in those cell culture and production facilities that do not have a designated area for microbiology work.

Our 100CFU™ Sensitivity Standards contain irreversibly inactivated mycoplasma in an amount corresponding to 100 CFU and provide an additional test concentration to the 10CFU™ Sensitivity Standards for the validation of PCR-based detection tests.

All strains are cultivated in low passages. Grown in culture medium as described in EP 2.6.7, the mycoplasma titer is determined after dilution in broth medium and cultivation on qualified mycoplasma agar plates. In order to ensure a high ratio of viable to non-viable mycoplasma and therefore a low ratio of genome units (GU) to CFU, the mycoplasma are harvested in the logarithmic growth phase. Genome copies are quantified after DNA extraction with Venor®GeM Sample Preparation Kit, using a Qubit® fluorometer. To ensure high confidence in the obtained results, qPCR with linear regression using PCR Quantification Standards is applied as a second quantification method.

TEST PRINCIPLE

Each vial contains a specific, irreversibly inactivated mycoplasma species in an amount corresponding to 100 CFU. Briefly, samples are prepared by adding the sample matrix.

Prior to PCR amplification, samples require DNA extraction. This is a necessary step in order to achieve maximum sensitivity, as shown in numerous round robin tests. DNA extraction can be easily performed using our Venor®GeM Sample Preparation Kit (Cat. No 56-1010/-1050/-1200). The extracted DNA shall be used directly for PCR.

Please note:
Due to the mycoplasma inactivation, the 100CFU™ Sensitivity Standards are not suitable for mycoplasma culture or culture-based methods. Please check the Certificate of Analysis for further lot-specific data. The GU:CFU ratio may vary between different lots.

CONTENT

Component	Quantity	NCTC codes	ATCC codes	Cat. No.
Acholeplasma laidlawii	3 vials	10116	23206	103-8003
Mycoplasma arginini		10129	23838	103-1003
Mycoplasma fermentans		10117	19989	103-6003
Mycoplasma gallisepticum		10115	19610	103-3003
Mycoplasma hyorhinis		10130	17981	103-7003
Mycoplasma orale		10112	23714	103-2003
Mycoplasma pneumoniae		10119	15531	103-4003
Mycoplasma salivarium		10113	23064	103-1103
Mycoplasma synoviae		10124	25204	103-5003
Spiroplasma citri		10164	27556	103-9003
Negative control	2 vials	n.a.	n.a.	n.a.

The date of expiry of the unopened product is given on the package label. The components must be stored until use at +2 to +8 °C. The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The product contains reagents for the preparation of samples spiked with inactivated mycoplasma particles. Additional general laboratory equipment or consumables (e.g. PCR reagents) need to be supplied by the user.
Required for EP 2.6.7/JP G3 compliant testing:

- 10CFU™ Sensitivity Standards available for all EP-/JP-listed mycoplasma species (see „Related Products“)
- DNA extraction kit, e.g. Venor®GeM Sample Preparation Kit (Cat. No. 56-1050)

RECOMMENDATIONS

⇨ We recommend using the 100CFU™ Sensitivity Standards strictly as described in the chapter „Procedure“. Any deviation from the test protocol may compromise the results.
⇨ We strongly advise against further dilution of the product (< 100 CFU/ml). Microorganisms particles often form complex clusters and inhomogeneous solutions with peculiar adherence properties. Dilution of such solutions may lead to significant loss or uneven distribution of the resuspended particles and unreliable measurements.
⇨ Avoid membrane-based microfiltration and ultracentrifugation (e.g. in the attempt of concentrating the samples) after addition of the 100CFU™ Sensitivity Standards. These procedures will result in considerable material loss and unreliable results.

PRECAUTIONS

The 100CFU™ Sensitivity Standards are for *in vitro* use only and should be applied by experienced laboratory staff. The products do not contain hazardous substances. However, all samples should be considered as potentially infectious and handled with all due care and attention. Always wear suitable lab coat and disposable gloves. Remaining material can be discarded according to local regulations.

ADDITIONAL NOTES

These instructions must be understood for a successful application of this product. The supplied reagents must not be mixed with reagents from different lots but used as an integral unit, only. The reagents must not be used beyond the expiry date.

PROCEDURE

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

1.	Spin down the vial(s) briefly.
2.	Add 1 ml of the sample matrix of interest to each vial.
3.	Incubate 5 min at room temperature.
4.	Vortex for 10 sec and spin down for 5 sec.
5.	Use an appropriate sample volume for DNA extraction. For DNA purification, we recommend our Venor®GeM Sample Preparation Kit.
6.	Use an appropriate volume of extracted DNA for PCR amplification.

In order to properly verify the results of the test, the negative controls must also be rehydrated with the sample matrix of interest and processed in parallel with the samples described above. An appropriate number of replicates is recommended. For the detection and analysis of the samples, we recommend our validated mycoplasma detection kits for conventional (Venor®GeM Classic Cat. No. 11-1025/-1050/-1100/-1250) or qPCR (Venor®GeM qEP Cat. No. 11-9025/-9100/-9250).

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

Qubit® a registered trademark of Thermo Fisher Scientific. Venor, Mynox, Onar, 100CFU, 10CFU and ZellShield are registered trademarks and Mycoplasma Off are trademarks of Minerva Biolabs GmbH.

Related Products

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*
102-1103	*Mycoplasma salivarium*
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 / JP listed species 2 vials per species, 10 CFU each

PCR Quantification Standards, 1 x 10^8 genomes / vial

52-0116	*Acholeplasma laidlawii*
52-0129	*Mycoplasma arginini*
52-0117	*Mycoplasma fermentans*
52-0115	*Mycoplasma gallisepticum*
52-0130	*Mycoplasma hyorhinis*
52-0112	*Mycoplasma orale*
52-0119	*Mycoplasma pneumonia*
52-0103	*Mycoplasma salivarium*
52-0124	*Mycoplasma synoviae*
52-0164	*Spiroplasma citri*

See MB homepage for further available species

Genomic DNA Extracts, 10 ± 2 ng / vial

51-0116	*Acholeplasma laidlawii*
51-0129	*Mycoplasma arginini*
51-0117	*Mycoplasma fermentans*
51-0115	*Mycoplasma gallisepticum*
51-0130	*Mycoplasma hyorhinis*
51-0112	*Mycoplasma orale*
51-0119	*Mycoplasma pneumonia*
51-0124	*Mycoplasma synoviae*
51-0164	*Spiroplasma citri*

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Contamination Control Kits for conventional PCR

11-1025/-1050/-1100/-1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 reactions
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 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
56-0002	Proteinase K	50 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

Mycoplasma Off™

15-1000/-5000	Surface Disinfectant Spray, spray bottle/refill bottle	1 l/5 l
15-1001/-5001	Surface Disinfectant Wipes in dispenser box/refill pack	50 wipes/5×50 wipes

ZellShield®

13-0050/-0150	Contamination Prevention Reagent 100× concentrate	50 ml/3×50 ml

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