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 nucleic acid amplification technology (NAT)-based mycoplasma tests.

The European Pharmacopoeia Chapter 2.6.7 (EP 2.6.7) and the Japanese Pharmacopoeia (17th Edition) Chapter G3 (JP G3) describe NAT-based mycoplasma tests such as PCR. Both require to show the detection of 10 Colony Forming Units per ml of sample volume (CFU/ml), if said tests 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, since most cell culture labs and production facilities do not have access to a microbiology lab, culturing or handling viable mycoplasma colonies to use as reference tools for sensitivity testing is inadmissible.

Our 10CFU™ Sensitivity Standards contain irreversibly inactivated mycoplasma in an amount corresponding to 10 CFU and allow safe and reliable validation.

All strains are cultivated in low passages. Grown in culture medium as described in EP 2.6.7, the 10CFU™ Sensitivity Standards are then titrated and plated for CFU determination. In order to ensure a high ratio of viable to non-viable mycoplasma and therefore a low ratio of GU* to CFU, the mycoplasma are harvested in the logarithmic growth phase.

*GU = genome units

TEST PRINCIPLE

Each vial contains a specific, irreversibly inactivated mycoplasma species in an amount corresponding to 10 CFU. Briefly, samples are prepared by adding the sample matrix of interest, as specified in EP 2.6.7 / JP G3.

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. The particular PCR test must show positive amplification for EP 2.6.7 / JP G3 compliance.

Please note:
Due to the mycoplasma inactivation, the 10CFU™ Sensitivity Standards are not suitable for mycoplasma culture or culture-based methods.
Our 10CFU™ Sensitivity Standards are prepared and quantified in terms of CFU numbers and not of genome copies, as EP 2.6.7 / JP G3 do 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 lots.

CONTENT

Component	Quantity	NCTC codes	ATCC codes	Cat. No.
Acholeplasma laidlawii	3 vials	10116	23206	102-8003
Mycoplasma arginini		10129	23838	102-1003
Mycoplasma fermentans		10117	19989	102-6003
Mycoplasma gallisepticum		10115	19610	102-3003
Mycoplasma hyorhinis		10130	17981	102-7003
Mycoplasma orale		10112	23714	102-2003
Mycoplasma pneumoniae		10119	15531	102-4003
Mycoplasma salivarium		10113	23064	102-1103
Mycoplasma synoviae		10124	25204	102-5003
Spiroplasma citri		10164	27556	102-9003
Negative control	2 vials	n.a.	n.a.	n.a.

The 10CFU™ Mycoplasma Set (Cat. No. 102-0002) contains 2 vials of each *Mollicutes* species listed in EP 2.6.7 (18 vials in total: *M. arginini, M. orale, M. gallisepticum, M. pneumoniae, M. synoviae, M. fermentas, M. hyorhinis, A. laidlawii, S. citri*) as well as 2 vials of the negative controls. *M. salivarium* is not included in the 10CFU™ Mycoplasma Set.

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 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.

PRECAUTIONS

The 10CFU™ Sensitivity Standards are for *in vitro* use only and should be applied by experienced laboratory staff. The products do not contain hazardous substances 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.

TEST 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. To meet further requirements in the context of the validation process, 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).

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. 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 for conventional PCR

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

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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PCR Cycler Validation

57-2102	PCR Cycler Check™ Advance	6 strips, 8 vials each
57-2103	PCR Cycler Check™ OneStep	100 reactions
57-2202	qPCR Cycler Check™	100 reactions

PCR Clean™

15-2025/15-2200/15-2500	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4×500 ml/5 l
15-2001	Wipes	120 wipes in a dispenser box
15-2002	Wipes, refill packs	5×120 wipes in a bag

Mycoplasma Off™

15-1000	Surface Disinfectant Spray, spray bottle	1 l
15-5000	Surface Disinfectant Spray, refill bottles	5 l
15-1001	Surface disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5×120 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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