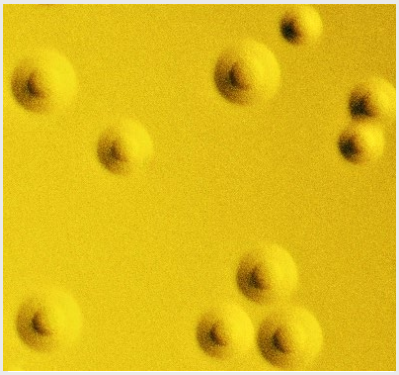
mycosafe®

Mycosafe® Inactivated Mycoplasma Culture Reference Standards

Validation Standards & Controls
for NAT-based Mycoplasma Detection Methods according to
EP 2.6.7., USP <63> & JP G3
for Application in Pharma QC, Cell & Gene Therapies, and Regenerative Medicine

17 Pharmacopoeia Mycoplasma Reference Strains



Titer Formats
1000-100-10 CFU per 100 µL

4 Product-Relevant Non-Pharmacopoeia Mycoplasma Reference Strains

User Guide

Low-GC/CFU
Inactivated Mid-log Phase Cell Preparations of Certified Mycoplasma Reference Strains

Intact Non-Viable Cells
Verified Growth Inability

October 2021

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Cover Images
10 single mid-log phase mycoplasma cells corresponding to 10 genome copies (GC) and 10 colony forming units (CFU), as confirmed for low-GC/CFU Mycosafe® Mycoplasma Culture Reference Standard preparations by a GC/CFU ratio of 1.
Mycosafe® Inactivated Mycoplasma Culture Reference Standards consisting of frozen mycoplasma cell preparations inactivated by heat treatment with confirmed non-viability, ensure a high level of laboratory and facility safety and are easy to use after a short thawing step by rapid warming.

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Mycosafe® Inactivated Mycoplasma Culture Reference Standards
Validation Standards & Controls for NAT- & Culture-based Mycoplasma Detection Methods according to EP 2.6.7., USP <63> & JP G3

User Guide
Low-GC/CFU Inactivated Mid-log Phase Cell Preparations of Certified Mycoplasma Reference Strains
Titer Formats 1000 - 100 - 10 CFU per 100 µL

October 2021

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1. Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products

This User Guide refers to the products of inactivated non-viable low-GC/CFU mycoplasma cell preparations listed below for use as a suitable alternative option to live mycoplasma cell preparations if internal safety regulations prohibit the handling of viable mycoplasma cells. These are easy-to-use frozen suspensions of intact non-viable culture-grown mid-log phase cells of all pharmacopoeia and important product-relevant non-pharmacopoeia mycoplasma reference strains and are provided as Mycosafe® Inactivated Single-Strain Reference Standards and convenient Mycosafe® Inactivated Multi-Strain Reference Standard Validation & Control Sets in low- and high-titer product formats. They are available in product units of 250 µL in cryovials. All Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products are traceable to authentic reference cultures of the National Collection of Type Cultures (NCTC), the American Type Culture Collection (ATCC), the European Directorate for the Quality of Medicines & HealthCare (EDQM) and the PHE Mycoplasma Reference Laboratory with Mycoplasma Experience Ltd as supply source, and are at low passage level that is ≤ 15 passages from the reference culture to comply with EP 2.6.7. (1), USP <63> (2) and JP G3 (3).

1.1 Mycosafe® Inactivated Single-Strain Culture Reference Standards

Mycosafe® Inactivated Single-Strain Culture Reference Standards
Product Formats

Product Format	1000-HI	100-HI	10-HI
Calibrated Total Cell Titer	1000 CFU/100 µL	100 CFU/100 µL	10 CFU/100 µL
Product Unit	250 µL	250 µL	250 µL
Mid-log Phase Mycoplasma Cells	●	●	●
Specified Low GC/CFU Ratio	●	●	●

Product/Order Numbers

Mycoplasma Reference Strain	Product/Order No.
Pharmacopoeia Type Strains (T)	
Acholeplasma laidlawii **PG8^T** NCTC 10116 ATCC 23206	Al-T-1000-HI Al-T-100-HI Al-T-10-HI
Mycoplasma arginini **G230^T** NCTC 10129 ATCC 23838	Ma-T-1000-HI Ma-T-100-HI Ma-T-10-HI
Mycoplasma fermentans **PG18^T** NCTC 10117 ATCC 19989	Mf-T-1000-HI Mf-T-100-HI Mf-T-10-HI
Mycoplasma gallisepticum **PG31^T** NCTC 10115 ATCC 19610	Mg-T-1000-HI Mg-T-100-HI Mg-T-10-HI
Mycoplasma hyorhinis **BTS7^T** NCTC 10130 ATCC 17981	Mh-T-1000-HI Mh-T-100-HI Mh-T-10-HI
Mycoplasma orale **CH19299^T** NCTC 10112 ATCC 23714	Mo-T-1000-HI Mo-T-100-HI Mo-T-10-HI
Mycoplasma pneumoniae **FH^T** NCTC 10119 ATCC 15531	Mp-T-1000-HI Mp-T-100-HI Mp-T-10-HI
Mycoplasma salivarium **PG20^T** NCTC 10113 ATCC 23064	Msa-T-1000-HI Msa-T-100-HI Msa-T-10-HI
Mycoplasma synoviae **WVU 1853^T** NCTC 10124 ATCC 25204	Ms-T-1000-HI Ms-T-100-HI Ms-T-10-HI
Spiroplasma citri **R8-A2^T** NCTC 10164 ATCC 27556	Sc-T-1000-HI Sc-T-100-HI Sc-T-10-HI

Mycoplasma Reference Strain	Product/Order No.
Pharmacopoeia Reference Field Strains (RFS)	
Acholeplasma laidlawii **RFS** EDQM Y0000693	Al-RFS-1000-HI Al-RFS-100-HI Al-RFS-10-HI
Mycoplasma fermentans **RFS** EDQM Y0000692	Mf-RFS-1000-HI Mf-RFS-100-HI Mf-RFS-10-HI
Mycoplasma hyorhinis **RFS** EDQM Y0000690	Mh-RFS-1000-HI Mh-RFS-100-HI Mh-RFS-10-HI
Mycoplasma orale **RFS** EDQM Y0000691	Mo-RFS-1000-HI Mo-RFS-100-HI Mo-RFS-10-HI
Mycoplasma synoviae **RFS** EDQM Y0000689	Ms-RFS-1000-HI Ms-RFS-100-HI Ms-RFS-10-HI
Mycoplasma pneumoniae **RFS 5167** Mycoplasma Experience	Mp-RFS-1000-HI Mp-RFS-100-HI Mp-RFS-10-HI
Pharmacopoeia 'Non-Cultivable' Cultivar α Reference Strain (Alpha)	
Mycoplasma hyorhinis **DBS 1050** ATCC 29052	Mh-Alpha-1000-HI Mh-Alpha-100-HI Mh-Alpha-10-HI

Mycoplasma Reference Strain	Product/Order No.
Product-Relevant Non-Pharmacopoeia Type Strains (T)	
Mycoplasma arthritidis **PG6^T** NCTC 10162 ATCC 19611	Mart-T-1000-HI Mart-T-100-HI Mart-T-10-HI
Mycoplasma bovis **PG45^T** NCTC 10131 ATCC 25523	Mb-T-1000-HI Mb-T-100-HI Mb-T-10-HI
Mycoplasma hominis **PG21^T** NCTC 10111 ATCC 23114	Mho-T-1000-HI Mho-T-100-HI Mho-T-10-HI
Ureaplasma urealyticum **T960^T** NCTC 10177 ATCC 27618	Uu-T-1000-HI Uu-T-100-HI Uu-T-10-HI

1.2 Mycosafe® Inactivated Multi-Strain Culture Reference Standard Validation & Control Sets

Categories of Mycosafe® Multi-Strain Reference Standard Validation & Control Sets	
EPUSP10	**All-in-One Validation Set according to EP 2.6.7. and USP <63>,** consisting of all 9 EP/USP mycoplasma type strains, including the type strains of *M. gallisepticum*, *M. synoviae* and *S. citri,* plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
EPUSP7	**Complete Basic Validation Set according to EP 2.6.7. and USP <63>,** consisting of all 6 non-avian and non-plant EP/USP mycoplasma type strains, plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
JP10	**All-in-One Validation Set according to JP G3.**, consisting of all 9 JP mycoplasma type strains including the type strains of *M. synoviae* and *S. citri,* plus the 'non-cultivable' *M. hyorhinis* cultivar α reference strain
JP7	**Complete Basic Validation Set according to JP G3**, consisting of all 7 non-avian and non-plant JP mycoplasma type strains
EPUSPJP5	**Small Basic Validation Set according to EP 2.6.7., USP <63> and JP G3.,** consisting of the 5 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. arginini, M. fermentans*, *M. hyorhinis* and *M. pneumoniae*
EPUSPJP3	**Control Set for Routine Mycoplasma Testing according to EP 2.6.7., USP <63> and JP G3,** consisting of the 3 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. orale* and *M. pneumoniae*
EP-EDQM5	**Complete Validation Set** of **all 5 EP EDQM mycoplasma reference field strains according to EP 2.6.7.**
EPUSP-RFS5	**Basic Validation Set of 5 mycoplasma reference field strains according to EP 2.6.7. and USP <63>**, including the 4 non-avian EP EDQM reference field strains and the *M. pneumoniae* reference field strain 5167 originating from the PHE Mycoplasma Reference Laboratory
ATMP8	**Complete Basic Validation Set for Blood- and Placenta Cell-derived Advanced Therapy Medicinal Products (ATPMs),** consisting of (i) the 6 EP/USP/JP mycoplasma type strains of *A. laidlawii, M. arginini, M. fermentans, M. orale, M. pneumoniae* and *M. salivarium*, and (ii) the 2 product-relevant non-pharmacopoeia mycoplasma type strains of *M. hominis* and *U. urealyticum*
ATMP9	**Extended Validation Set for Blood- and Placenta Cell-derived ATMPs,** consisting of the same mycoplasma reference strain combination as comprising the ATMP Complete Basic Set, plus the non-pharmacopoeia type strain of *M. bovis* that is required if bovine serum is used during the manufacturing process

Mycosafe® Inactivated Multi-Strain Culture Reference Standard Validation & Control Sets
Product Formats

Product Format	1000-HI	100-HI	10-HI
Calibrated Total Cell Titer	1000 CFU/100 µL	100 CFU/100 µL	10 CFU/100 µL
Product Unit	250 µL	250 µL	250 µL
Mid-log Phase Mycoplasma Cells	●	●	●
Specified Low GC/CFU Ratio	●	●	●

Product/Order Numbers

Mycoplasma Reference Standard Strain Set Category	Product/Order No.
EPUSP10	EPUSP10-1000-HI
	EPUSP10-100-HI
	EPUSP10-10-HI
EPUSP7	EPUSP7-1000-HI
	EPUSP7-100-HI
	EPUSP7-10-HI
JP10	JP10-1000-HI
	JP10-100-HI
	JP10-10-HI
JP7	JP7-1000-HI
	JP7-100-HI
	JP7-10-HI

Mycoplasma Reference Standard Strain Set Category	Product/Order No.
EPUSPJP5	EPUSPJP5-1000-HI
	EPUSPJP5-100-HI
	EPUSPJP5-10-HI
EPUSPJP3	EPUSPJP3-1000-HI
	EPUSPJP3-100-HI
	EPUSPJP3-10-HI

Mycoplasma Reference Standard Strain Set Category	Product/Order No.
EP-EDQM5	EP-EDQM5-1000-HI
	EP-EDQM5-100-HI
	EP-EDQM5-10-HI
EPUSP-RFS5	EPUSP-RFS5-1000-HI
	EPUSP-RFS5-100-HI
	EPUSP-RFS5-10-HI
ATMP8	ATMP8-1000-HI
	ATMP8-100-HI
	ATMP8-10-HI
ATMP9	ATMP9-1000-HI
	ATMP9-100-HI
	ATMP9-10-HI

2. Scope of Use

Mycosafe® Inactivated Mycoplasma Culture Reference Standards are frozen inactivated low- and high-titer mycoplasma cell preparations for use in

(i) quality control of biopharmaceuticals and cell & gene therapy products as **Validation Standards** and as **External Controls** in NAT-based mycoplasma testing applications, and

(ii) laboratory *in vitro* research purposes.

Mycosafe® Inactivated Mycoplasma Culture Reference Standards are not for any human or animal therapeutic or diagnostic use.

3. Intended Use

Mycosafe® Inactivated Mycoplasma Culture Reference Standards are intended for use as reference material and controls in in-process and release mycoplasma testing by **NAT-based mycoplasma detection methods** according to EP 2.6.7. (1), USP <63> (2) and JP G3 (3). They are frozen low-GC/CFU mycoplasma reference standard products at low and high titer that are particularly designated for use in direct Real-Time PCR testing **if safety regulations prevent the handling of live mycoplasma cell preparations**.

Mycosafe® Inactivated Mycoplasma Culture Reference Standards provide an easy-to-use suitable alternative to live mycoplasma cell preparations, if the use of viable mycoplasmas is due to contamination risks within the laboratory facilities prohibited. As according to the mycoplasma NAT validation guidelines in EP 2.6.7. (1) and JP G3 (3) "the complete procedure from extraction of nucleic acid to detection of the amplified products" must be considered for validation of an analytical method, the use of inactivated low-GC/CFU mycoplasma culture reference standards as Validation Standards is a suitable alternative. Samples spiked with Mycosafe® Inactivated Mycoplasma Culture Reference Standards may simulate like samples spiked with live mycoplasma cell preparations a 'real' contamination, with the need to overcome the challenge of mycoplasma DNA recovery at low spike levels corresponding to low contamination levels.

For the generic and product-specific validation of NAT-based mycoplasma detection methods, such as the currently commercially available Real-Time PCR mycoplasma detection systems, Mycosafe® Inactivated Mycoplasma Culture Reference Standards are therefore appropriate for use as **Validation Standards**, if Mycosafe® Mycoplasma Culture Reference Standards in the viable product format can due to facility or laboratory safety regulations not be used. Likewise, Mycosafe® Inactivated Mycoplasma Culture Reference Standards are appropriate for use as **External Controls** during routine application of validated Real-Time PCR mycoplasma test methods.

Mycosafe® Inactivated Mycoplasma Culture Reference Standards cannot be used for the culture-based mycoplasma testing methods, such as the Culture Method and Indicator Cell Culture Method according to EP 2.6.7., USP <63> and JP G3 Likewise, Mycosafe® Inactivated Mycoplasma Culture Reference Standards cannot be used for the Real-Time PCR Hybrid Method that involve a short enrichment step in liquid culture medium prior to Real-Time PCR analysis.

4. Composition & Specifications

Mycosafe® Inactivated Mycoplasma Reference Standards are comprised of non-viable intact mid-log phase cells of certified mycoplasma reference strains suspended at low or high titer in FRIIS, FREY, Mycosafe® ABO™ or Mycosafe® UREA™ medium. The irreversibly inactivated products were prepared by suitable heat treatment, and the resulting non-viability of the mycoplasma cells was verified by growth controls.

Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products are supplied frozen in product units of 250 µL aliquots contained in 1.5- or 2-mL plastic cryovials.
The verified non-viability of each Mycosafe® Inactivated Mycoplasma Culture Reference Standard Product, the mycoplasma species identity, the traceability to an authentic reference culture of a specified mycoplasma reference strain from the NCTC, the ATCC, the EDQM or from the PHE Mycoplasma Reference Laboratory with Mycoplasma Experience Ltd as supply source, as well as the low passage level from the reference culture, the calibrated CFU titer of the total mycoplasma cells and the low GC/CFU ratio are stated in a corresponding **lot-specific Certificate of Analysis (CoA)**.

5. Materials for Optional Use but not Provided

Diluent
Particularly for the high-titer product format (1000-S-HI), a diluent may be required to obtain the desired concentration of inactivated total mycoplasma cells that is suitable for the intended use.
For the preparation of diluted inactivated mycoplasma cell suspensions, the use of Phosphate-Buffered Saline (molecular biology grade PBS) as diluent is recommended.

6. Instructions for Use

6.1 Preparation for Use

Rapid Thawing
(i) For thawing, the frozen inactivated mycoplasma reference standard material is rapidly warmed at 36 ± 1°C (< 1 minute in a water bath or heating block) until there is just a small bit of ice left in the cryovial, and then kept on ice prior to use for direct spike inoculation without further dilution, or for preparation of an appropriate working dilution.

Optional Preparation of Working Dilutions
(ii) After rapid thawing of the frozen inactivated mycoplasma reference standard material at 36 ± 1°C it may be - if required or desired - diluted with molecular biology grade PBS to obtain the desired number of inactivated total mycoplasma cells in an appropriate spike inoculation volume that is suitable for the intended use.
(iii) The final dilution and the required dilution steps must be based on the titer of the total mycoplasma cell number, which is specified in the corresponding CoA in CFU.
(iv) The dilution steps with the PBS diluent must be carried out on ice immediately after thawing of the frozen inactivated mycoplasma reference standard material.
(v) The diluted inactivated mycoplasma reference standard material must be kept on ice if not immediately used.

Spike Inoculation
(vi) The product sample material to be spiked must be ready for spike inoculation as soon as the inactivated mycoplasma reference standard material is ready to be used in the desired total cell concentration – either undiluted or in the desired working dilution.
(vii) The ready-to-use undiluted or diluted inactivated mycoplasma reference standard material must be mixed prior to use by vortexing the vial for 10 seconds at full speed to obtain an optimal homogeneous suspension of the inactivated mycoplasma cells
(viii) The spike inoculation with the ready-to-use undiluted or properly diluted suspension of inactivated mycoplasma cells should be completed within 2 hours following the thawing step.

6.2 Spike Inoculation Doses

Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products are designed for single, few, several or multiple spike inoculations at the same time, depending on the titer format and unit size, the target sample spike level and the applied spiking procedure (direct spiking of test sample replicates, or spiking of a larger test sample volume prior to aliquoting into identical test sample replicates), including the volume of the product sample material and the number of sample replicates to be spiked. Examples for different Spike Inoculation Doses per mL test sample replicate are given below.

Product Format		1000-HI	100-HI	10-HI
Spike Inoculation Dose 100 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*		10 µL	100 µL	
Number Doses per Product Unit* **	Single (1-5)	●	●	
	Few (6-25)	●		
	Several (26-100)			
	Multiple (> 100)			
Spike Inoculation Dose 10 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*		1 µL	10 µL	100 µL
Number Doses per Product Unit* **	Single (1-5)	●	●	●
	Few (6-25)	●	●	
	Several (26-100)	●		
	Multiple (> 100)	●		
Spike Inoculation Dose 1 CFU Total Cells per mL Test Sample				
Spike Inoculation Volume Size Range per Dose Undiluted Ready-to-Use*			1 µL	10 µL
Appropriate Dilution Required for Spike Inoculation**		●		
Number Doses per Product Unit* **	Single (1-5)	●	●	●
	Few (6-25)	●	●	●
	Several (26-100)	●	●	
	Multiple (> 100)	●	●	

*The exact volume of spike inoculum needs to be individually calculated for each test or test series
**To be determined
***Undiluted or diluted

6.3 Repeated Use after Storage & Disposal

(i) The remaining unused undiluted and diluted inactivated mycoplasma reference standard material may be either stored at ≤ - 60°C for further single use (see under Section 7 below) or disposed by autoclaving (see under Section 8 below). For long-term storage and multiple use, it is recommended to divide the undiluted or diluted inactivated mycoplasma cell preparations into several aliquots, each for separate use as required.
(ii) Typically, the low-titer product formats (100-S-HI and 10-S-HI) are used only once. The remaining unused undiluted or diluted inactivated mycoplasma reference standard material is therefore usually disposed immediately.

6.4 Summary of Instructions for Use

(i) If necessary, determining the working dilution required to obtain the desired spike concentration for the product sample material to be spiked, particularly when using the higher-titer product format 1000-S-HI.
(ii) Removing the cryovial containing the undiluted frozen inactivated mycoplasma reference standard material from the ≤ -60°C freezer.
(iii) Rapid thawing of the frozen material by warming at 36 ± 1°C (< 1 min) and keeping it on ice after thawing.
(iv) Conducting the required dilution steps - if required - on ice using molecular biology grade PBS as diluent kept on ice.
(v) Keeping the ready-to-use undiluted or diluted inactivated mycoplasma reference standard material on ice if not immediately used.

(vi) Mixing the ready-to-use undiluted or diluted inactivated mycoplasma reference standard material prior to use by vortexing the vial for 10 seconds at full speed.
(vii) Having the product sample material to be spiked ready for spike inoculation with the ready-to-use undiluted or diluted inactivated mycoplasma reference standard material.
(viii) Completing the spike inoculation with the undiluted or properly diluted suspension of inactivated mycoplasma cells **within 2 hours following the thawing step**.
(ix) Storing the unused undiluted or diluted inactivated reference standard material at ≤ - 60°C for further single use or disposing by autoclaving.

6.5 Additional Notes

(i) For successful use of Mycosafe® Inactivated Mycoplasma Culture Reference Standards, they should not be used beyond their shelf life indicated in the corresponding CoA.
(ii) Deviations from the described Instructions for Use may affect the results.
See also under Section 9 below.

7. Storage & Stability

The frozen Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products have a high stability during long-term storage at ≤ -60°C.

Once received, they must be stored properly at ≤ - 60°C prior to thawing for use up to the expiration date specified in the corresponding lot-specific CoA. Long-term exposure to higher temperatures than ≤ - 60°C can adversely affect the stability of the inactivated mycoplasma reference standard material.

After use for spike inoculation, the rest of the undiluted or diluted inactivated mycoplasma reference standard material may be stored at ≤ - 60°C for further single use.

If the inactivated mycoplasma reference standard material, particularly the higher-titer product format 1000-S-HI, is diluted and not immediately used after dilution, users should determine the shelf life of the diluted preparations according to their own assessment based on experience, method of preparation, storage conditions and use. When stored at ≤ - 60°C, a shelf life of 1 year for single use from the date of preparation, i.e., the date of dilution, is recommended to be issued for the freshly prepared diluted inactivated mycoplasma reference standard material.

8. Precautions

Mycosafe® Inactivated Mycoplasma Culture Reference Standards consist of irreversibly inactivated but intact mycoplasma cells. The validated heat inactivation step was carried out at an optimum temperature and exposure time. As this mycoplasma reference standard material is irreversibly inactivated, it is not regarded as infectious and has therefore the Biosafety Level 1. General laboratory precautions and good laboratory practise should however be employed when handling Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products, by wearing proper personal protective equipment, such as an appropriate laboratory coat, protective eye wear and disposable gloves.

Mycosafe® Inactivated Mycoplasma Culture Reference Standards are to be used by trained laboratory personnel only, that has experience in the handling of infectious biological material. Even though this mycoplasma reference standard material has been inactivated and tested for innocuousness, it should be treated with the same degree of care as if it contains viable mycoplasma cells and is potentially infectious.

It is therefore recommended that Mycosafe® Inactivated Mycoplasma Culture Reference Standards should be handled in accordance with Biosafety Level 2 practices as described in corresponding guidelines. Consequently, all application procedures should be handled in a microbiology safety cabinet conforming to EN12469:2000 Biotechnology - Performance Criteria for Microbiological Safety Cabinets. Hand-to-mouth contact should be avoided while working with the inactivated mycoplasma reference standard material, and normal handwashing and disinfection procedures relating to the handling of infectious biological material should be observed also with the non-infectious heat-inactivated mycoplasma reference standard material.
Users of Mycosafe® Inactivated Mycoplasma Culture Reference Standards assume all risk and responsibility in connection with the receipt, handling, storage, disposal, transfer and use of these products. Refer to the **Material Safety Data Sheet (MSDS)** for more detailed information.

Disposal Measures
Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products do not contain any hazardous substances. The remaining unused material or the remaining unused material derived therefrom by dilution must be - unless not stored at ≤ -60°C for further single use (see under Section 7 above) - disposed by using an autoclave as for samples containing infectious microorganisms
(i) in accordance with the implemented laboratory protocol for disposal of biohazard materials, and
(ii) in accordance with all national and local regulations.

9. Warranty & Limitations

Mycosafe® Inactivated Mycoplasma Culture Reference Standards are warranted to meet the specifications given in the CoAs and related Mycosafe® Product Information Material. The warranty, expressed or implied, is limited and the stated characteristics and specifications in the corresponding CoAs cannot be guaranteed when
(i) Mycosafe® Inactivated Mycoplasma Culture Reference Standards are employed for applications other than the intended use described in this User Guide and cited in the Mycosafe® Mycoplasma Reference Standards Product Flyer,
(ii) the procedures employed are different to the directions and instructions given in this User Guide,
(iii) Mycosafe® Inactivated Mycoplasma Culture Reference Standards are repeatedly thawed and refrozen,
(iv) Mycosafe® Inactivated Mycoplasma Culture Reference Standards are stored improperly at higher temperatures than ≤ - 60°C, and
(v) Mycosafe® Inactivated Mycoplasma Culture Reference Standards are used after the expiration date has passed.

Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products are supplied with the condition that users are responsible for their safe storage, handling, and use. Prof Rosengarten and Mycosafe® Consulting assume no liability for any direct, indirect, consequential, or incidental damages resulting from the use, the results of use, or the inability to use Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products.

10. Restrictions

Mycosafe® Inactivated Mycoplasma Culture Reference Standards contain trade secrets and intellectual property of Prof Renate Rosengarten and may not be modified, copied or replicated by users, or not be transferred by users to any third party in whole or in part.

The rights granted to the user by purchasing Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products are personal to the user. The user may not resell or distribute Mycosafe® Inactivated Mycoplasma Culture Reference Standard Products or any part thereof in any way.

11. References

(1) European Pharmacopoeia, 10th Edition, Chapter 2.6.7., Mycoplasmas, 2020.
(2) United States Pharmacopoeia, 43rd Edition, Chapter 63, Mycoplasma Tests, 2020.
(3) Japanese Pharmacopoeia, 18th Edition, Mycoplasma Testing for Cell Substrates used for the Production of Bio-technological/Biological Products, in: Chapter G3 Biotechnological/Biological Products, 2021.

12. User Guide at a Glance

Mycosafe® Inactivated Single-Strain Culture Reference Standards	**21 Mycoplasma Reference Strains** • 10 pharmacopoeia type strains (EP 2.6.7., USP <63>, JP G3) • 6 pharmacopoeia reference field strains (EP 2.6.7., USP <63>) • 1 pharmacopoeia 'non-cultivable' cultivar α reference strain (EP 2.6.7., USP <63>, JP G3) • 4 product-relevant non-pharmacopoeia type strains • Traceability to authentic reference cultures of the NCTC, the ATCC, the EDQM, and Mycoplasma Experience Ltd • Strain passage level: P1 to ≤ P15 • Confirmed low GC/CFU ratio: usually 1-5, can be slightly higher for some Mycosafe® Inactivated Mycoplasma Culture Reference Standard lots of the pharmacopoeia type strains of *Mycoplasma fermentans, Mycoplasma pneumoniae*, *Mycoplasma salivarium, Mycoplasma synoviae* and *Spiroplasma citri,* and the reference field strain 5167 of *Mycoplasma pneumoniae* • Intact non-viable mycoplasma cells inactivated by heat treatment at an optimum temperature and exposure time • For detailed information, see the Mycosafe® Reference Standards Product Flyer
Mycosafe® Inactivated Multi-Strain Culture Reference Standard Validation & Control Sets	**10 Strain Set Categories** • EPUSP10, EPUSP7, EP-EDQM5, EPUSP-RFS5 • JP10, JP7 • EPUSPJP5, EPUSPJP3 • ATMP8, ATMP9 For detailed information, see the Mycosafe® Reference Standards Product Flyer
Product/Titer Formats & Product Unit Size	**Product Formats**: • 1000-HI • 100-HI • 10-HI **Calibrated Total Cells/100 µL**: • 1000 • 100 • 10 **Product Unit Size**: • 250 µL For detailed information, see the Mycosafe® Reference Standards Product Flyer
Scope of Use	• Validation Standards, and External/Positive Controls in NAT-based mycoplasma testing methods for quality control of biopharmaceuticals and cell & gene therapy products • Laboratory *in vitro* research purposes
Intended Use	• Intended for use as Validation Standards and External Controls for in-process and release mycoplasma testing by NAT-based mycoplasma detection methods according to EP 2.6.7., USP <63> and JP G3, if the use of live mycoplasmas is due to contamination risks within the laboratory facilities prohibited
Compositon & Specifications	• Cultured mid-log phase cells of certified mycoplasma reference strains suspended at low or high CFU titer in FRIIS, FREY, Mycosafe® ABO™ or Mycosafe® UREA™ medium, which have been inactivated by heat treatment as verified by growth controls • Supplied frozen in product units of 250µL aliquots contained in 1.5- or 2-mL plastic cryovials • Specifications stated in lot-specific Certificates of Analysis (CoAs): (i) the mycoplasma species identity; (ii) the traceability to an authentic reference culture of a certified mycoplasma reference strain from NCTC, ATCC, EDQM, or from Mycoplasma Experience Ltd; (iii) the passage level from the original reference culture; (iv) the calibrated CFU titer of the total mycoplasma cells; (v) the GC/CFU ratio; (vi) the verified non-viability
Materials for Optional Use but not Provided	• Phosphate-Buffered Saline (molecular biology grade PBS) as optional diluent particularly for the high-titer product format (1000-S-HI) to obtain the desired spike inoculation doses of inactivated total mycoplasma cells that are suitable for the intended use
Instructions for Use	• Rapid thawing of the frozen inactivated mycoplasma reference standard material at 36 ± 1°C (< 1 min) • If required, conducting suitable dilution steps on ice using molecular biology grade PBS as diluent to obtain the desired working dilution • Keeping the ready-to-use undiluted or diluted inactivated mycoplasma reference standard material on ice if not immediately used • Mixing the ready-to-use undiluted or diluted inactivated mycoplasma reference standard material prior to use by vortexing the vial for 10 seconds at full speed • Completing the spike inoculation with the undiluted or properly diluted suspension of inactivated mycoplasma cells within 2 hours following the thawing step • The unused undiluted or diluted inactivated mycoplasma reference standard material may be stored at ≤ - 60°C for further single use, or disposed by autoclaving

Storage & Stability	• High stability during long-term storage at ≤ -60°C • After application, the remaining undiluted or diluted inactivated mycoplasma reference standard material may be stored at ≤ - 60°C for further single use • If the inactivated mycoplasma reference standard material, particularly the high-titer product format, is diluted and not immediately used after dilution, users should determine the shelf life of the diluted preparation according to their own assessment based on experience, method of preparation, storage conditions and use. When stored at ≤ - 60°C, a shelf life of 1 year for single use from the date of preparation, i.e., the date of dilution, is recommended to be issued for the freshly prepared diluted mycoplasma reference standard material
Precautions	• Mycosafe® Inactivated Mycoplasma Culture Reference Standards do not contain any hazardous substances and have the Biosafety Level 1, but should be treated with the same degree of care as if they contain viable mycoplasma cells and are potentially infectious • General laboratory precautions and good laboratory practise for Biosafety Level 2 should therefore be employed, as described in corresponding guidelines: (i) Handling by trained laboratory personnel only, that has experience in the handling of infectious biological material and wears proper personal protective equipment; (ii) handling of all application procedures in a microbiology safety cabinet conforming to EN12469:2000 Biotechnology - Performance Criteria for Microbiological Safety Cabinets; (iii) avoiding hand-to-mouth contact while working with the mycoplasma reference standard material; (iv) normal handwashing and disinfection procedures relating to the handling of infectious biological material • The remaining unused inactivated mycoplasma reference standard material or the remaining used or unused material derived therefrom by dilution must be - unless not stored at ≤ -60°C for further single use - disposed by using an autoclave as for samples containing infectious microorganisms and in accordance with all national and local regulations • For detailed information, see the Mycosafe® Mycoplasma Reference Standards Material Safety Data Sheet (MSDS) for inactivated mycoplasma cell preparations
Warranty & Limitations	• Product warranty is limited to the characteristics and specifications stated in the corresponding CoA • The stated characteristics and specifications cannot be guaranteed (i) for applications other than the intended use, (ii) when the procedures employed are different to the given directions and instructions, (iii) when repeated thawing/freezing cycles are applied, (iv) when higher temperatures than ≤ - 60°C are applied for long-term storage, (v) when the expiration date has passed
Restrictions	• Product changes, copies and replications as well as the transfer, resale and distribution in whole or in part by users to third parties are prohibited

Related Mycosafe® Products, Consultancy & Licensing Options

1. Alternative Mycosafe® Reference Standard Product Formats

Mycosafe® Viable Mycoplasma Culture Reference Standards
Total Cell Titer: 1000, 100, 10 CFU/100 µL
Product Unit: 250 µL

Mycosafe® Mycoplasma DNA Reference Standards
Titer: 100 GC/µL
Product Unit: 100 µL

For detailed information, refer to the Mycosafe® Reference Standards Product Flyer

2. Mycosafe® Mycoplasma Expert Consultancy

Scientific, Regulatory- & Business-Related Mycoplasma Expert Advisory Support Options
Risk Assessments & Mycoplasma Safety Concepts: Biopharmaceuticals · ATMPs · Raw Materials

For detailed information, please contact Prof Rosengarten at renate.rosengarten@mycosafe-consulting.com

3. Mycosafe® Licensing Options

Mycosafe® ABO™ Universal Mycoplasma Rapid Enrichment Medium
for Real-Time PCR Hybrid Assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Non-commercial product licensing options

Mycosafe® UREA™ Ureaplasma Rapid Enrichment Medium
for Real-Time PCR Hybrid Assays according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Commercial & non-commercial product licensing options

Mycosafe® ALPHA™ Selective Medium
for 'non-cultivable' cultivar α strains according to the forthcoming revised Mycoplasma Testing Guidelines of EP 2.6.7.
Commercial & non-commercial product licensing options

Mycosafe® SF™ Serum-Free Mycoplasma Culture Media
for preparation of mycoplasma antigens
Commercial & non-commercial product licensing options

Mycosafe® Mycoplasma Images
Selection of high-quality authentic mycoplasma images for any marketing project related to products for mycoplasma detection and culture- and NAT-based mycoplasma testing solutions
Exclusive, semi-exclusive & non-exclusive image licensing options

Mycosafe® Know-How
End-to-End Rapid Mycoplasma Testing Solutions
Commercial & non-commercial know-how licensing options

For detailed information, please contact Prof Rosengarten at renate.rosengarten@mycosafe-consulting.com

Contact for Further Information

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