SARTORIUS

Instructions for Use

Cyclus® 100 GC

SMB95-3001	Cyclus® 100 GC *Mycoplasma arginini*
SMB95-3002	Cyclus® 100 GC *Mycoplasma orale*
SMB95-3003	Cyclus® 100 GC *Mycoplasma gallisepticum*
SMB95-3004	Cyclus® 100 GC *Mycoplasma pneumoniae*
SMB95-3005	Cyclus® 100 GC *Mycoplasma synoviae*
SMB95-3006	Cyclus® 100 GC *Mycoplasma fermentans*
SMB95-3007	Cyclus® 100 GC *Mycoplasma hyorhinis*
SMB95-3008	Cyclus® 100 GC *Acholeplasma laidlawii*
SMB95-3009	Cyclus® 100 GC *Spiroplasma citri*
SMB95-3010	Cyclus® 100 GC *Mycoplasma salivarium*

Validation Standard and External Positive Control
For use in research and quality control

Symbols

LOT	Lot No.
REF	Order No.
	Expiry date
	Store at
	Content
	Manufacturer

Contents

1. Intended Use

Cyclus® 100 GC are intended for the validation of nucleic acid amplification technology (NAT)-based mycoplasma detection methods in accordance with the requirements of the European Pharmacopoeia (EP) 2.6.7 Issue 12.2, as well as in alignment with current NAT concepts described in USP <77> (draft) and JP 18 G3.

The standards provide defined, non-infectious nucleic acids for spiking product-specific matrices to determine assay sensitivity (LOD) and robustness as required for pharmacopeial method validation.

Cyclus® 100 GC may also be used as low-concentration external positive controls (EPC) in routine NAT-based testing.

Depending on the workflow and pharmacopeial framework, they can additionally serve as extraction-based controls when added prior to nucleic acid extraction (e.g., corresponding to the extraction inhibition control EIC described in USP <77> draft) or as positive PCR controls (PPC) when added directly to the amplification reaction.

The standards are suitable exclusively for molecular NAT methods and are not intended for culture-based mycoplasma assays.

2. Test Principle

Cyclus® 100 GC provide purified, non-infectious mycoplasma genomic nucleic acids at a defined concentration of 100 genome copies (GC) per vial. The material is intended to be spiked into product-specific sample matrices to evaluate the performance of NAT-based mycoplasma detection methods.

When added to the matrix of interest, the standards allow users to assess the sensitivity of their overall workflow, typically consisting of nucleic acid extraction followed by PCR.

The standards can be applied for:
- LOD determination during product-specific method validation, and
- use as an EPC, EIC or/and PPC in routine testing, either processed according to the complete validated workflow (including extraction), or applied directly as a low-concentration PCR positive control without prior extraction.

Because the standards contain isolated genomic nucleic acids and no viable organisms, they are suitable exclusively for molecular detection methods and do not support culture-based assays.

3. Reagents

Cyclus® 100 GC are provided at a defined concentration of 100 genome copies (GC) per vial. Each lot is quantified by digital PCR (dPCR). Lot-specific information is provided in the accompanying Quality Assurance Certificate.

Component	Prod. No.	Quantity	Cap color
Mycoplasma arginini	SMB95-3001	3 vials <100 GC with DNA/RNA (lyophilized)	green
Mycoplasma orale	SMB95-3002		
Mycoplasma gallisepticum	SMB95-3003		
Mycoplasma pneumoniae	SMB95-3004		
Mycoplasma synoviae	SMB95-3005		
Mycoplasma fermentans	SMB95-3006		
Mycoplasma hyorhinis	SMB95-3007		
Acholeplasma laidlawii	SMB95-3008		
Spiroplasma citri	SMB95-3009		
Mycoplasma salivarium	SMB95-3010		
PCR grade Water	Included in all products	1 vial	white

Storage and Stability (unopened product):

- Store at +2 °C to +8 °C.
- The expiry date of the unopened product is indicated on the package label.

After Reconstitution / Rehydration:
Each vial is intended for single use only.
Rehydrated material must not be stored or reused.

Exception:
If the standard is reconstituted in PCR grade Water, the following storage conditions apply:

- 2-8 °C for up to 7 days, or
- ≤-18 °C for up to 3 months.

When mixed with sample matrix or lysis buffer plus matrix, the material should be used immediately, as storage stability is matrix-dependent and cannot be guaranteed.

The LOT-specific Quality Assurance Certificate can be downloaded from the MySartorius portal (https://my.sartorius.com).

4. Needed but not included

Cyclus® 100 GC require only basic laboratory consumables for reconstitution and handling.

All additional materials depend on the NAT-based detection method used downstream and must be provided by the user.

Consumables

- RNase-free, DNase-free low-bind tubes (1.5 mL or 5 mL)
- RNase-free, DNase-free pipette filter tips (100 – 1000 µL)
- Personal protective equipment (laboratory gloves, protective masks)
- Sartorius Cleaning Spray (SMB95-5001/SMB95-5002) or Cleaning Wipes (SMB95-5003/SMB95-5004) for contamination control
- PCR reaction tubes, strips or plates suitable for the chosen amplification platform

Laboratory equipment

- Pipettes (Suitable pipettes and corresponding filter tips are available from Sartorius)
- Vortex mixer
- Microcentrifuge for 1.5 mL tubes
- Suitable PCR cycler
- Tube racks and general laboratory equipment applicable to the chosen detection method

Recommended extraction and detection systems

For optimal performance and to ensure recovery of both DNA and RNA, the following systems are recommended:

- Cyclus® Bead Extraction (SMB95-6000)
- Cyclus® RT-qPCR Mycoplasma (SMB95-6002)

5. Sample

Cyclus® 100 GC are intended for spiking into product-specific sample matrices requiring mycoplasma testing. The standards themselves do not require a specific matrix composition. Instead, each user must evaluate whether their own product-specific matrix is compatible with the chosen nucleic acid extraction and NAT-based amplification method.

Applicable sample types

The standards can be used with all matrices typically evaluated in NAT-based mycoplasma testing, including:

- cell culture supernatants
- suspension and adherent cell cultures
- viral vector preparations (e.g., AAV, LV, adenovirus)
- plasmid-, DNA- or mRNA-based products
- upstream and downstream processing intermediates
- formulated drug substances and drug products
- cryopreservation media and supplements
- buffer systems, process intermediates and stabilizers

Depending on product type and regulatory expectations, testing may be required for both cellfree and cell-containing samples.

Matrix considerations

Many matrices contain DNases and/or RNases, which may degrade nucleic acids. To prevent loss of genomic material, follow the handling instructions provided in this manual:

- Protocol 1: For nuclease-free matrices
- Protocol 2: For matrices with potential nuclease activity
- Protocol 3: For direct PCR testing / low-concentration EPC or PPC

Highly viscous or hydrophobic matrices may impair dissolution or rehydration of the standard. In such cases, ensure thorough mixing and evaluate whether additional steps are required to achieve complete homogenization.

Once mixed with a matrix, or with lysis buffer plus matrix, storage stability becomes matrix dependent, and no general stability claims can be made. Immediate use is recommended.

Use in method validation

The standards support matrix-specific validation of NAT-based mycoplasma detection methods. The specific validation design (e.g., number of product batches, organism selection, representative matrices) must be defined by the user based on regulatory requirements and internal quality guidelines.

Matrices used for validation should represent the final product or the relevant manufacturing stage, as defined in the validation plan.
This document does not define a complete validation protocol. Such procedures must comply with applicable regulatory requirements, including EP 2.6.7, USP <77>, JP 18 G3, and internal risk-based assessments.

Matrix Suitability Testing

The "Method Suitability Test" (USP <77>) and the "Test for Inhibitory Substances" (EP 2.6.7) are required to demonstrate that the user's product-specific matrix does not interfere with nucleic acid extraction or PCR amplification.

For this purpose, Cyclus® 100 GC may be spiked into the matrix and processed through the complete NAT workflow. Successful detection confirms that the matrix supports efficient extraction and amplification without inhibitory effects.

6. Precautions

Cyclus® 100 GC are intended for in vitro use only and must be handled by experienced laboratory personnel following good laboratory practice.

The product contains purified, non-infectious genomic nucleic acids and does not pose a biological infection risk. Nevertheless, all samples and spiked matrices should be treated as potentially infectious.

Always wear appropriate personal protective equipment, including a lab coat, disposable gloves, and a protective mask, to avoid sample contamination and exposure.

PCR carry-over contamination may lead to false-positive results. Use dedicated pipettes, RNase-/DNase-free consumables, and contamination control reagents such as Sartorius Cleaning Spray (SMB95-5001/SMB955002) or Cleaning Wipes (SMB95-5003/SMB95-5004).

Avoid repeated freeze-thaw cycles and do not use the product beyond its expiry date.

The standards are not suitable for culture-based mycoplasma assays, as they contain no viable organisms.

Dispose of unused materials and consumables according to local laboratory waste regulations.

7. Additional Notes

1. Matrix handling and nuclease considerations

- If the matrix is nuclease-free, rehydrate the standard directly in the matrix (Protocol 1).
- If the matrix contains DNases and/or RNases (e.g., fetal calf serum, cell lysates), rehydrate the standard in lysis buffer before adding it to the matrix (Protocol 2).
- Once the standard has been mixed with matrix (or with lysis buffer + matrix), stability cannot be guaranteed; immediate processing is recommended.
- Highly viscous or hydrophobic matrices may impair rehydration; ensure thorough mixing.

2. Use as controls in NAT-based mycoplasma testing

- Extraction inhibition control (before extraction)
  When spiked into the matrix before nucleic acid extraction, Cyclus® 100 GC can be used to verify extraction efficiency and matrix compatibility within NAT workflows (e.g., as described in USP <77> draft).
- Positive PCR control (direct amplification)
  When added directly to the PCR reaction, the standard serves as a low-copy PCR control to verify PCR performance independently of extraction.
- External Positive Control (EPC)
  When used according to the validated NAT workflow, Cyclus® 100 GC may serve as a low-concentration external positive control (EPC).
  It can be added before nucleic acid extraction or directly to the PCR reaction, depending on whether the validated method includes an extraction step.
  In both cases, the EPC verifies overall assay performance close to the detection limit and confirms that the complete workflow functions as intended.

3. Direct PCR use

- For direct PCR testing or use as a low-concentration external positive control, the standard may be rehydrated in PCR grade Water (Protocol 3).
- When reconstituted in PCR grade Water, the material may be stored for 7 days at +2 °C to +8 °C or 3 months at ≤ –18 °C.

4. General laboratory handling

- Use aerosol-resistant filter tips and change tips between pipetting steps to prevent carryover contamination.
- Reagents must not be mixed with components from other lots and must not be used beyond their expiry date.
- Any deviation from the instructions in this document may affect assay performance and must be validated by the user.
- These instructions must be fully understood and carefully followed for successful application of this product.

8. Test Procedure

Protocol 1 – Nuclease-free matrices

1.	Centrifuge the vial briefly for 5 sec at maximum speed.
2.	Add 1 mL of the product-specific matrix to the vial.
3.	Incubate for at least 5 min. **Note:** Solubility depends on the matrix. For challenging matrices, extend incubation (e.g., 15 min) and/or increase the temperature up to 65 °C to support rehydration.
4.	Mix thoroughly by pulse-vortexing (3 × 10 sec). **Note:** Avoid foam formation. If necessary, mix by gentle pipetting without drawing air.
5.	Centrifuge briefly to collect any liquid from the lid.
6.	Proceed with analysis according to the instructions of the selected NAT-based mycoplasma detection method.

Protocol 2 – Nuclease-containing matrices

1.	Centrifuge the vial briefly for 5 sec at maximum speed.
2.	Add 1 mL of lysis buffer (Cyclus® Bead Extraction SMB95-6000). Additional lysis buffer available as **Cyclus® Bead Extraction Lysis Buffer** (SMB95-6003) if needed. **Note:** When using extraction systems from other manufacturers, adjust volumes according to their instructions.
3.	Incubate for at least 5 min.
4.	Mix thoroughly by pipetting at least 10 times, avoiding air intake.
5.	Add the entire 1 mL rehydrated standard to 1 mL of the product-specific matrix to generate 4 technical replicates.
6.	Mix thoroughly again by pipetting at least 10 times.
7.	Centrifuge briefly to collect any liquid from the lid.
8.	Aliquot 500 µL per extraction (corresponding to 250 µL sample).
9.	Add 20 µL Proteinase K (from the Cyclus® Bead Extraction (SMB95-6000)) per extraction.
10.	Perform extraction according to the instructions of the Cyclus® Bead Extraction (SMB95-6000)

Note on Internal Control (IC):
For workflows using an internal control as a process control, add the IC to the lysis buffer before rehydrating the standard. For the Cyclus® RT-qPCR Mycoplasma (SMB95-6002), add 6.4 µL IC to 1 mL lysis buffer (based on a 100 µL IC stock).

Protocol 3 – Direct testing / low-concentration EPC or PPC

1.	Centrifuge the vial briefly for 5 sec at maximum speed.
2.	Add 100 µL PCR grade Water.* **Note:** This results in a concentration of 1 GC/µL. Adjust other concentrations by increasing the volume accordingly.
3.	Incubate for at least 5 min. **Note:** Rehydration may be improved by extending incubation or increasing temperature (e.g., 65 °C for 10 min).
4.	Mix thoroughly by pulse-vortexing (3 × 10 sec). **Note:** Avoid foam formation. If necessary, mix by gentle pipetting without drawing air.
5.	Use the standard directly in reaction mix.

*Recommendation: For PCR without Reverse Transcriptase step, rehydrate in 100 µL PCR grade Water. For Reverse Transcriptase PCR, rehydrate in 100 µL or 1000 µL PCR grade Water.

Recommended detection systems

To meet regulatory and validation requirements in NAT-based mycoplasma testing, Cyclus® RT-qPCR Mycoplasma (SMB95-6002) detection kit is recommended and validated with CyclusR Bead Extraction (SMB95-6000).

This kit combination is optimized for sensitive and reliable NAT-based mycoplasma detection and is fully compatible with the Cyclus® 100 GC.

9. Related Products

Detection Kits for qPCR or dPCR

SMB95-6001	Cyclus® dPCR Tool Box Bacteria Fungi	10 samples
SMB95-6002	Cyclus® RT-qPCR Mycoplasma	25 tests
SMB95-1007	Microsart® ATMP Sterile Release	10 samples
SMB95-1008	Microsart® ATMP Bacteria	100 tests
SMB95-1009	Microsart® Research Bacteria	25 tests
SMB95-1012	Microsart® ATMP Fungi	100 tests
SMB95-1014/1013	Microsart® Research Fungi	25/100 tests

Cyclus® 100 GC , 3 vials

SMB95-3001	Cyclus® 100 GC *Mycoplasma arginini*
SMB95-3002	Cyclus® 100 GC *Mycoplasma orale*
SMB95-3003	Cyclus® 100 GC *Mycoplasma gallisepticum*
SMB95-3004	Cyclus® 100 GC *Mycoplasma pneumoniae*
SMB95-3005	Cyclus® 100 GC *Mycoplasma synoviae*
SMB95-3006	Cyclus® 100 GC *Mycoplasma fermentans*
SMB95-3007	Cyclus® 100 GC *Mycoplasma hyorhinis*
SMB95-3008	Cyclus® 100 GC *Acholeplasma laidlawii*
SMB95-3009	Cyclus® 100 GC *Spiroplasma citri*
SMB95-3010	Cyclus® 100 GC *Mycoplasma salivarium*

Cyclus® 10 CFU, 3 vials

SMB95-3011	Cyclus® 10 CFU *Mycoplasma arginini*
SMB95-3012	Cyclus® 10 CFU *Mycoplasma orale*
SMB95-3013	Cyclus® 10 CFU *Mycoplasma gallisepticum*
SMB95-3014	Cyclus® 10 CFU *Mycoplasma pneumoniae*
SMB95-3015	Cyclus® 10 CFU *Mycoplasma synoviae*
SMB95-3016	Cyclus® 10 CFU *Mycoplasma fermentans*
SMB95-3017	Cyclus® 10 CFU *Mycoplasma hyorhinis*
SMB95-3018	Cyclus® 10 CFU *Acholeplasma laidlawii*
SMB95-3019	Cyclus® 10 CFU *Spiroplasma citri*
SMB95-3020	Cyclus® 10 CFU *Mycoplasma salivarium*

Microsart® Calibration Reagent, 10^8 genomes / vial, 1 vial (bacteria)

SMB95-2030	*Bacillus subtilis*
SMB95-2031	*Pseudomonas aeruginosa*
SMB95-2032	*Kocuria rhizophila*
SMB95-2033	*Clostridium sporogenes*
SMB95-2034	*Bacteroides vulgatus*
SMB95-2035	*Staphylococcus aureus*
SMB95-2036	*Mycoplasma salivarium*

Microsart® Calibration Reagent, 10^6 genomes / vial, 1 vial (fungi)

SMB95-2044	*Candida albicans*
SMB95-2045	*Aspergillus brasiliensis*
SMB95-2046	*Aspergillus fumigatus*
SMB95-2047	*Penicillium chrysogenum*
SMB95-2048	*Candida glabrata*
SMB95-2049	*Candida krusei*
SMB95-2050	*Candida tropicalis*

Microsart® Validation Standard, 99 CFU / vial, 6 vials each (bacteria and fungi)

SMB95-2005	*Bacillus subtilis*
SMB95-2006	*Pseudomonas aeruginosa*
SMB95-2007	*Kocuria rhizophila*
SMB95-2008	*Clostridium sporogenes*
SMB95-2009	*Bacteroides vulgatus*
SMB95-2010	*Staphylococcus aureus*
SMB95-2037	*Candida albicans*
SMB95-2038	*Aspergillus brasiliensis*
SMB95-2039	*Aspergillus fumigatus*
SMB95-2040	*Penicillium chrysogenum*
SMB95-2041	*Candida glabrata*
SMB95-2042	*Candida krusei*
SMB95-2043	*Candida tropicalis*

DNA Extraction Kit

SMB95-6000	Cyclus® Bead Extraction (for mollicutes)	100 extractions
SMB95-6003	Cyclus® Bead Extraction Lysis Buffer	27.5 mL
SMB95-2001	Microsart® ATMP Extraction (for bacteria and fungi)	50 extractions
SMB95-4000	Microsart® Proteinase K	50 extractions

Cleaning Spray

SMB95-5001	DNA Decontamination Reagent, spray bottle	250 mL
SMB95-5002	DNA Decontamination Reagent, refill canister	5 L

Cleaning Wipes

SMB95-5003	DNA Decontamination Reagent, wipes	50 wipes
SMB95-5004	DNA Decontamination Reagent, refill sachets	5 × 50 wipes

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 out of the use, the results of use, or the inability to use this product.

Trademarks

Cyclus and Microsart are registered trademarks of Sartorius.

Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Str. 20
37079 Goettingen, Germany

Phone +49 551 308 0
Fax +49 551 308 3289
www.sartorius.com

Status:
March 2026,
Sartorius,
Goettingen, Germany

Publication No.: SM-3001-e260302
Ver. 03 | 2026