SARTORIUS

Instructions for Use

# Cyclus® RT-qPCR Mycoplasma

Prod. No. SMB95-6002

Reagents for 25 reactions
For use in research and quality control

Symbols

| | |
|---|---|
| LOT | Lot No. |
| REF | Order No. |
|  | Expiry date |
|  | Store at |
|  | Contains reagents for 25 reactions |
|  | Manufacturer |

# Contents

# 1. Intended Use

Cyclus® RT-qPCR Mycoplasma is designed for the rapid and highly sensitive detection of *Mycoplasma, Acholeplasma,* and *Spiroplasma,* in cell and gene therapy medicinal products, also called Advanced Therapy Medicinal Products (ATMPs), cell cultures, and other biological matrices.

The assay is designed as a nucleic acid amplification technique (NAT)-based method and meets the test criteria of the European Pharmacopoeia (EP) 2.6.7 Issue 12.2, the Japanese Pharmacopoeia (JP) 18 G3, and the United States Pharmacopeia (USP) <63> and USP <77>.

The validated kit has been tested with a variety of real-time PCR instruments and offers consistent and reliable performance. It delivers a rapid and accurate alternative to culture-based methods, which may require up to 28 days, and does not depend on viable mycoplasma cells, ensuring robust detection of both culturable and non-culturable organisms.

The method achieves sensitivities of ≤10 CFU/mL and <100 GC/mL, supporting regulatory compliant testing of diverse sample types with high reproducibility.

# 2. Test Principle

The Cyclus® RT-qPCR Mycoplasma detects mycoplasma by amplifying a highly conserved region of the 16S rRNA and its coding sequence using a real-time PCR workflow. The target region is conserved across all relevant species listed in EP 2.6.7 Issue 12.2, JP 18 G3, USP <63>, USP <77> (see section "Assay Characteristics"). The assay does not amplify eukaryotic DNA or non-mollicute bacteria, ensuring high specificity.

Extraction of nucleic acids is required to achieve optimal sensitivity and to remove potential PCR inhibitors. This step is essential for reliable detection in diverse sample matrices used in ATMP and biopharmaceutical manufacturing, including cell culture supernatants, serum-containing media, cryopreservation solutions, and formulation buffers.

The workflow includes a reverse transcription step, enabling detection of both DNA and RNA forms of Mollicutes. This increases sensitivity and ensures robust detection of culturable and non-culturable mycoplasmas.

Mycoplasma-specific targets (assay target and Positive Control) are detected via a FAM™-labeled probe (fluorescence at 520 nm). The Internal Control target is detected via a HEX™-labeled probe (fluorescence at 560 nm; HEX™, VIC®, or JOE™ channel compatible).

The Internal Control (IC) monitors nucleic acid extraction efficiency, reverse transcription, and PCR amplification performance. It can be added either to the sample before extraction to serve as an extraction and in-process control, or to the PCR master mix as an amplification control.

The workflow is validated in combination with the Cyclus® Bead Extraction (SMB95-6000), a magnetic bead-based nucleic acid extraction system optimized for the efficient recovery of mycoplasma DNA and RNA from complex biopharmaceutical and ATMP-relevant matrices. This combined workflow ensures regulatory-compliant NAT-based detection with high sensitivity and specificity.

# 3. Reagents

Each kit provides reagents for 25 reactions. The expiry date of the unopened kit is indicated on the outer package label. Kit components must be stored at +2 °C to +8 °C until rehydration. After rehydration, the Mycoplasma Mix must be stored at ≤-18 °C and used within 30 days. Internal Control (IC) and Positive Control (PC) can be stored at ≤-18 °C until the end of their specified shelf life. To ensure the integrity of the reagents, repeated freeze-thaw cycles must be avoided.

| **Kit component** | **Quantity**<br>**25 Reactions**<br>**Prod. No. SMB95-6002** | **Cap color** |
|---|---|---|
| Mycoplasma Mix | 1 vial, lyophilized | red |
| Positive Control | 1 vial, lyophilized | green |
| Internal Control | 1 vial, lyophilized | yellow |
| PCR grade Water | 2 vials, 2 mL | white |

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

# 4. Needed but not included

Cyclus® RT-qPCR Mycoplasma contains all the reagents to perform the test. General industrial supplies and reagents, usually available in PCR laboratories, are not included:

- qPCR device with filter sets for detecting the fluorescent dyes FAM™ and HEX™ (or alternatively filter sets for detecting VIC® oder JOE™)
- PCR reaction tubes, strips or plates and caps, recommended by the manufacturer of your thermal cycler
- Microcentrifuge for 1.5 mL reaction tubes
- 1.5 mL reaction tubes, DNase- and RNase-free (preferably low-bind)
- Pipettes (Suitable pipettes and corresponding filter tips are available from Sartorius)
- Nucleic acid extraction kit, e.g. Cyclus® Bead Extraction (Prod. No. SMB95-6000)

**For process control or EP-/JP-/USP-compliant testing:**

- Cyclus® 100 GC and Cyclus® 10 CFU available for all EP-/JP-/USP-listed mycoplasma species (see table below or „Related Products" section)

**For contamination control:**

- Chlorine-based cleaning agent
- Sartorius Cleaning Spray (SMB95-5001/SMB95-5002) or Cleaning Wipes (SMB95-5003/SMB95-5004)

**Optional:**

- 10 mM Tris buffer, pH 8.4

| Species | Prod. No. | |
|---|---|---|
| | Cyclus® 100 GC | Cyclus® 10 CFU |
| *Mycoplasma arginini* | SMB95-3001 | SMB95-3011 |
| *Mycoplasma orale* | SMB95-3002 | SMB95-3012 |
| *Mycoplasma gallisepticum* | SMB95-3003 | SMB95-3013 |
| *Mycoplasma pneumoniae* | SMB95-3004 | SMB95-3014 |
| *Mycoplasma synoviae* | SMB95-3005 | SMB95-3015 |
| *Mycoplasma fermentans* | SMB95-3006 | SMB95-3016 |
| *Mycoplasma hyorhinis* | SMB95-3007 | SMB95-3017 |
| *Acholeplasma laidlawii* | SMB95-3008 | SMB95-3018 |
| *Spiroplasma citri* | SMB95-3009 | SMB95-3019 |
| *Mycoplasma salivarium* | SMB95-3010 | SMB95-3020 |

# 5. Important Notes

## 5.1 Handling recommendations to avoid false-positive results

Because the assay is highly sensitive, strict contamination control measures are required. To avoid false positive results due to improper handling the following actions are recommended:

1. Always wear a clean laboratory coat, disposable gloves, and a medical face mask. The use of single-use arm sleeves is recommended to minimize the risk of contamination from clothing or exposed skin. Personal protective equipment should be changed regularly, particularly when moving between pre-PCR and post-PCR areas.
2. All work must be conducted according to good laboratory and manufacturing practice and standard PCR contamination control procedures. A strict physical separation of workflow steps is strongly recommended, with dedicated areas for sample preparation, nucleic acid extraction, PCR reaction mix preparation, and post-PCR analysis.
3. Perform the test under sterile and DNA-free conditions, the use of a laminar flow cabinet is recommended.
4. The laminar flow cabinet and all materials introduced into the laminar flow cabinet should be thoroughly decontaminated with a chlorine-based cleaning agent before and during the work process.
5. Avoid working above open tubes and avoid air turbulences due to rapid movements.
6. Be careful when opening the tubes. Do not touch the inner surface of the lid. Close the lid immediately after each pipetting step.
7. Use only aerosol-resistant filter tips and regularly clean work surfaces with suitable decontamination reagents.
8. Decontamination of work areas using Sartorius Cleaning Spray (SMB95-5001/SMB95-5002), Cleaning Wipes (SMB95-5003/SMB95-5004) or chlorine-based cleaning agent is recommended to further reduce the risk of amplicon contamination.
9. Avoid opening PCR tubes or plates after amplification.

According to EP 2.6.7 Issue 12.2, the inclusion of specific controls is mandatory. Each run must contain a No Template Control (NTC), a Negative Extraction Control (NEC), an Internal Control (IC), and an External Positive Control (EPC) to verify sensitivity, extraction performance, amplification efficiency, and the absence of contamination. In addition, it is strongly recommended to include the kit-internal Positive Control (PC) in every run. While not required by the pharmacopeia, the PC provides an additional verification of PCR reagent integrity and supports robust troubleshooting, helping to ensure reliable results and safety.

## 5.2 Explanation of Controls

**Positive Control (PC):** A synthetic DNA construct used to verify the correct performance of the PCR reaction. It confirms that the assay reagents, thermocycler, primers, and probes for the target gene are functioning as expected. The PC cannot assess extraction efficiency.

**No Template Control (NTC):** PCR grade Water or elution buffer included in the PCR run to detect reagent contamination.

**Negative Extraction Control (NEC):** An extraction performed using PCR grade Water or a known negative sample. It monitors contamination introduced during the extraction process or handling.

**Internal Control (IC):** The IC included in Cyclus® RT-qPCR Mycoplasma must be used to verify nucleic acid extraction efficiency, the reverse transcription step, and overall PCR performance. To prevent degradation by RNases present in many biological matrices, the IC must be added together with the lysis buffer or immediately after its addition.

For use as an extraction and in-process control, the IC should be reconstituted in 100 µL PCR grade Water. This concentrated preparation keeps spike-in volumes low and prevents unnecessary dilution of the sample matrix. Based on internal validation data, a spike-in of 6.4 µL IC per 1 mL sample provides optimal performance. This amount yields IC Ct values in extracted samples comparable to the Ct values obtained in No Template Controls (NTC) containing 1 µL IC reconstituted in 1000 µL, ensuring a balanced IC signal without impairing assay sensitivity.

When the IC is used only as an assay control, it should be reconstituted in 1000 µL PCR grade Water or prepared as a 1:10 dilution of the concentrated IC used for extraction controls. This ensures appropriate fluorescence intensity while avoiding competition with target amplification.

The IC input should be adjusted according to the expected final elution volume to ensure a consistent Internal Control concentration in the eluate. Lower IC spike-in volumes may be sufficient depending on matrix validation results, whereas excessively high IC amounts can reduce assay sensitivity due to competition between IC and target amplification.

**Important:** If the IC is added before extraction, do not add it to the PCR master mix.

**External Positive Control (EPC):**
EPC is an additional positive control used independently of the detection kit. According to EP 2.6.7 (12.2), it must contain a defined number of target-sequence copies or CFUs and is set close to the cut-off, to demonstrate that the validated sensitivity is achieved.

**Extraction Inhibition Control (EIC):**
EIC is an additional positive control used independently of the detection kit. According to USP <77>, it must contain a defined number of target-sequence copies or CFUs and is set close to the cut-off and have to be spiked into the matrix before nucleic acid extraction, to demonstrate extraction efficiency and that the validated sensitivity is achieved. In this manual, the term EPC is used throughout. The EIC serves the same functional purpose; therefore, references to EPC also apply to EIC.

**Note:** The EPC and EIC are not included in the kit. For EPC and EIC the use of Cyclus® 100 GC or Cyclus® 10 CFU is recommended**.** Detailed instructions for EPC use are provided in the respective product manuals.

# 6. Precautions

Cyclus® RT-qPCR Mycoplasma is intended for in vitro use only and must be handled by trained laboratory personnel following established molecular biology procedures. All samples must be regarded as potentially infectious and handled accordingly.

The kit does not contain any hazardous substances according to applicable regulations. All remnants and biological samples must be disposed of in compliance with local biosafety and waste management guidelines.

# 7. Additional Notes

Carefully read and fully understand these instructions before using Cyclus® RT-qPCR Mycoplasma. All reagents must be used as a complete unit and must not be mixed with reagents from other kit batches. Do not use reagents beyond the indicated shelf life.

Follow the protocol exactly as described. Any deviation may affect assay performance, sensitivity, and result validity.

PCR inhibition is often caused by product-specific sample matrices. Therefore using Cyclus® Bead Extraction (SMB95-6000) for sample preparation is recommended. Any alternative nucleic acid extraction method must be validated by the user.

Extracted nucleic acids should be processed immediately or stored at ≤-18 °C to maintain stability and ensure reliable amplification.

Freeze–thaw cycles of samples should be avoided, as they may affect nucleic acid integrity and reduce assay sensitivity.

Control samples must be processed in the same manner as test samples to ensure accurate interpretation.

To minimize the risk of carry-over contamination, prepare and handle Positive Controls only after completing all negative controls and test reactions.

Rehydrate the Mycoplasma Mix according to the instructions in the "Procedure" section and dispense the required volume directly into PCR tubes or strips. Use the rehydrated mix immediately or store it at ≤-18 °C and use it within 30 days.

When testing cell culture samples, collect material when cell cultures have reached approximately 80–90% confluence.

Common antibiotics (e.g. penicillin, streptomycin) and cell culture additives do not inhibit mycoplasmas and do not interfere with assay sensitivity. Any change in the sample matrix, extraction method, or sample preparation workflow must be revalidated by the user to ensure that the required sensitivity and PCR performance are maintained.

# 8. Sample Preparation

Nucleic acid extraction is mandatory to achieve the required analytical sensitivity and to reliably remove inhibitors that may be present in product-specific matrices. Using Cyclus® Bead Extraction (SMB95-6000), which has been validated in combination with Cyclus® RT-qPCR Mycoplasma (SMB95-6002) provides regulatory-compliant extraction performance.

Follow the instructions for use of Cyclus® Bead Extraction for the sample preparation.

## 8.1 PCR testing of extracts

Each extract must be tested in the PCR. Extracted nucleic acids should be stored at 4 °C and processed within 24 h or stored at ≤-18 °C to preserve nucleic acid integrity.

## 8.2 Validation requirements

Any alternative extraction method or modified workflow must be validated by the user to demonstrate suitability for the specific sample matrix and compliance with EP 2.6.7 sensitivity requirements.

# 9. Test Procedure

## 9.1 Reagent preparation

The test should be carried out with internal, negative and external positive controls and samples in appropriate numbers.

**Note:** After rehydration, the Mycoplasma Mix must be stored at ≤-18 °C and used within 30 days. The Internal Control and Positive Control may be stored at ≤-18 °C until the end of their specified shelf life. Avoid repeated freeze–thaw cycles to preserve reagent integrity. Reconstituted reagents should be aliquoted immediately to prevent performance loss during storage.

| | Used components | Cap colors | Steps |
|---|---|---|---|
| 1. | Mycoplasma Mix<br>Internal Control<br>Positive Control | red cap<br>yellow cap<br>green cap | Spin down all lyophilized components for 5 sec at maximum speed. |
| 2. | Mycoplasma Mix<br>PCR grade Water | red cap<br>white cap | Add 265 µL PCR grade Water to Mycoplasma Mix. |
| 3. | Internal Control<br>PCR grade Water | yellow cap<br>white cap | Rehydrate the IC with 100 µL PCR grade Water.* |
| 4. | Positive Control<br>PCR grade Water | green cap<br>white cap | Rehydrate the PC with 400 µL PCR grade Water. |
| 5. | Mycoplasma Mix<br>Internal Control<br>Positive Control | red cap<br>yellow cap<br>green cap | Vortex briefly and incubate at least 10 min at room temperature.** |
| 6. | Mycoplasma Mix | red cap | Vortex briefly and spin for 5 sec. |
| 7. | Internal Control<br>Positive Control | yellow cap<br>green cap | Vortex thoroughly and spin for 5 sec. |

* This concentrated IC preparation is recommended for all applications. Depending on the intended use, dilute the rehydrated IC accordingly:

- In-process control: use the IC directly as rehydrated in 100 µL PCR grade Water.
- Assay control: prepare a 1:10 dilution of the IC reconstituted in 100 µL PCR grade Water.

** Increasing the incubation temperature of the Positive Control to 60 °C can have a positive effect on the final result.

## 9.2 Reaction mix preparation

Follow these schemes and sequences to set up the test. Consider a specific order of PCR reactions, for example start with NTC, followed by samples, EPC and PC, to prevent cross contamination.

1. Homogenize the prepared Mycoplasma Mix by pipetting at least 5-times up and down before pipetting 10 µL Mycoplasma Mix into each PCR reaction tube.
2. Prepare **NTC**: Add 10 µL PCR grade Water or Elution Buffer supplied with Cyclus® Bead Extraction. Add 1 µL of diluted IC to NTC PCR. Cap the NTC PCR tubes.
3. Prepare **sample**: Add 10 µL of nucleic acid extract. Cap the sample PCR tubes. *
4. Prepare **EPC**: Add 10 µL of External Positive Control (extract). Cap the EPC PCR tubes. *
5. Prepare **PC**: If possible, move to a separate environment where only the PC is processed with dedicated equipment. Add 10 µL of rehydrated Positive Control. Add 1 µL of diluted IC to PC PCR tubes. Cap the PC PCR tubes.
6. Ensure that all PCR reaction tubes are closed tightly and spin them down briefly.

* If IC was not introduced during nucleic acids extraction, 1 µL of 1:10 diluted IC should be added to the PCR reaction mix.

## 9.3 PCR amplification

1. Place PCR strips in the qPCR device and close the lid.
2. Program the qPCR cycler or check stored temperature profiles.

| | |
|---|---|
| 1 cycle | 50 °C for 40 min |
| 1 cycle | 95 °C for 2 min |
| 44 cycle | 95 °C for 15 sec |
| | 59 °C for 1 min & read data |

3. Start the program.

# 10. Data interpretation

The presence of mycoplasma is indicated by an increasing fluorescence signal in the FAM™ channel. The determination of Ct values, including baseline settings and normalization, depends on the specific qPCR instrument and its software. Please refer to the instrument documentation for detailed instructions.

All amplification curves, including controls, should be visually inspected to confirm proper reaction performance and overall data quality.

A PCR reaction is considered positive when a mycoplasma-specific signal appears in the FAM™ channel with a Ct value below 40. Reactions with Ct values of 40 or higher are considered negative. When the Internal Control is added, a valid PCR run is indicated by an increasing signal in the FAM™ channel, the HEX™ channel, or both. Depending on the qPCR platform, the Internal Control may also be detected in the VIC® or JOE™ channel.

The assay is based on competitive amplification. High levels of mycoplasma nucleic acid result in a strong FAM™ signal and a reduced Internal Control signal. The absence of mycoplasma nucleic acid leads to a strong IC signal in HEX™ / VIC® / JOE™ channel with no amplification in the FAM™ channel. This design supports simultaneous target detection and internal validation of each reaction.

The External Positive Control must produce a clearly detectable signal to confirm that the assay performs with the required sensitivity. Due to the wide range of possible EPC materials and concentrations, no universal target Ct range can be provided. The selection of a suitable EPC and the establishment of internal acceptance criteria are the responsibility of the user and must be defined as part of the product-specific method validation.

The following table contains general information on interpreting PCR results:

| **FAM™ channel – Deection of Mollicutes** | **HEX™ / VIC® / JOE™ channel – Internal Control Detection** | **Interpretation** |
|---|---|---|
| positive | irrelevant | Positive for Mollicutes |
| negative | negative | PCR inhibition |
| negative | positive | Negative for Mollicutes |

# 11. Troubleshooting Guide

This troubleshooting guide provides solutions to common issues that may occur during sample preparation, RT-qPCR setup, or result analysis.

| Issue | Cause and solution |
|---|---|
| Insufficient mycoplasma lysis | **Cause**: The lysis buffer may not be optimal, or incubation time/temperature was insufficient.<br>**Solution:** Verify that the lysis buffer is used according to protocol. Ensure that incubation steps are carried out exactly as described. If viscosity is high, pipette slowly and avoid air bubbles. |
| Magnetic bead carry-over | **Cause:** Incomplete separation during nucleic acid purification using magnetic beads.<br>**Solution:** After placing tubes on the magnetic rack, wait the full recommended time until the solution is clear. Carefully pipette without disturbing beads. Avoid drawing beads into the eluate, as this can inhibit PCR. |
| RNA degradation | **Cause:** Exposure to RNases or prolonged storage at inappropriate temperatures.<br>**Solution:** Use only RNase-free consumables and reagents. For storage, freeze eluates immediately at -18 °C in low-binding tubes, and avoid repeated freeze-thaw cycles. |
| Lower-than-expected mycoplasma signal | **Cause:** Incomplete RNA extraction or inefficient RT-PCR reaction.<br>**Solution:** Use validated extraction kits optimized for both RNA and DNA (e.g. Cyclus® Bead Extraction (SMB95-6000)). Make sure no inhibitors are present and that the reaction mix is freshly prepared before use. Increase sample input if necessary. |
| No positive signal | **Cause:** The target concentration may be below the detection limit, or the PCR settings may be incorrect.<br>**Solution:** Verify that all assay components are used correctly and that the cycling conditions match the protocol. Ensure the correct fluorescence channels are activated in the instrument software, and confirm that no unnecessary reference channels are enabled.<br>If sensitivity is insufficient, note that the PCR reaction volume cannot be increased. Only the sample volume prior to extraction can be increased, provided that the extraction workflow remains compatible with validation requirements. If appropriate for the sample type, the sample may be concentrated by centrifugation and reduction of the supernatant volume to increase the amount of target nucleic acid entering the extraction process. |

| | |
|---|---|
| Low Internal Control signal | **Cause:** Improper rehydration or degradation of the Internal Control, or extraction failure.<br>**Solution:** Rehydrate the lyophilized IC exactly as described in the protocol and mix thoroughly. Ensure that the IC is added either during lysis (for in-process control) or directly into the RT-PCR master mix (for amplification control). Verify that the extraction procedure was performed correctly.<br>**Note:** When the IC is used as an in-process control, a low signal does not allow discrimination between poor recovery and PCR inhibition. This can be easily verified by retesting the extract and adding the IC directly to the master mix. A normal IC signal in this repeat test indicates reduced extraction efficiency, whereas a persistently low signal indicates inhibition. |
| Incorrect or unsuitable threshold setting in PCR software | **Cause:** Automatic baseline and threshold may be inappropriate for certain runs or instruments.<br>**Solution:** After each run, check the automatically set baseline and threshold and adjust them manually if necessary using validated criteria (e.g. fixed value, percentage of max fluorescence of a Positive Control, or inflection point of a standard curve). |

# 12. Assay Characteristics

## 12.1 Sensitivity

The detection limit is determined using the EP 2.6.7-, JP 18 G3-, USP <63>- and USP <77>-compliant workflow, including nucleic acid extraction and the mandatory assay controls. The assay reliably achieves the required analytical sensitivity of <100 GC/mL or ≤10 CFU/mL.

For validation, all pharmacopeia-listed mycoplasma species were spiked into cell culture medium (DMEM with 10 % FCS), extracted using Cyclus® Bead Extraction, and subsequently analysed with the Cyclus® RT-qPCR Mycoplasma. All validation data are documented in our validation report, which is available upon request.

The following table lists all mycoplasma species referenced in the pharmacopeial guidelines that were used for LOD determination.

| Species | Detection limit $LOD_{95}$ | Detection limit $LOD_{95}$ |
|---|---|---|
| *Mycoplasma arginini* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma orale* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma gallisepticum* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma pneumoniae* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma synoviae* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma fermentans* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma hyorhinis* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Acholeplasma laidlawii* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Spiroplasma citri* | <100 [GC/mL] | ≤10 [CFU/mL] |
| *Mycoplasma salivarium* | <100 [GC/mL] | ≤10 [CFU/mL] |

## 12.2 Specificity

The specificity of Cyclus® RT-qPCR Mycoplasma was evaluated according to the requirements of EP 2.6.7 by testing for the absence of cross-reactivity with the non-mollicute microorganisms listed in the pharmacopeia. No amplification was observed for *Streptococcus, Lactobacillus,* or *Clostridium* under the recommended assay conditions.

The microorganisms and mammalian species included in the specificity assessment are listed in the table below.

| Microorganisms | Mammals |
|---|---|
| *Bacillus subtilis* | Vero |
| *Bacteroides vulgatus* | HL-60 |
| *Burkholderia cepacia* | HEK-293 |
| *Clostridium acetobutylicum* | CHO K1 |
| *Clostridium perfringens* | |
| *Clostridium sporogenes* | |
| *Kocuria rhizophila* | |
| *Lactobacillus acidophilus* | |
| *Pseudomonas aeruginosa* | |
| *Staphylococcus aureus* | |
| *Streptococcus equinus* | |
| *Streptococcus pneumoniae* | |

# 13. 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.

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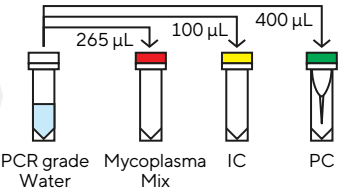
# 14. Short Instruction

## 1. Reagent preparation

Mycoplasma Mix, IC and PC:
briefly




briefly
≥10 min RT

Mycoplasma Mix:
briefly
for 5 sec

IC and PC:
thoroughly
for 5 sec

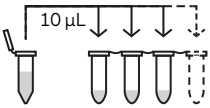
## 2. Reaction mix preparation

**Reaction mix for n reactions**

10 µL Mycoplasma Mix × n
1 µL IC* × n

Homogenize ≥5 times

Aliquot 10 µL of prepared reaction mix into each PCR strip

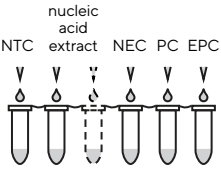



Discard remaining material

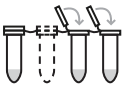
## 3. Addition of Samples and Controls

**10 µl reaction mix**

+ 10 µL elution buffer (NTC) or
+ 10 µL nucleic acid extract or
+ 10 µL negative extraction control (NEC) or
+ 10 µL PC or
+ 10 µL External Positive Control (EPC)

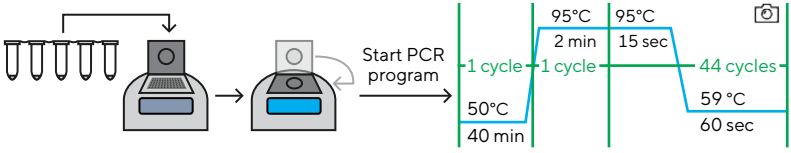



Close tightly



briefly

## 4. PCR amplification




- Mycoplasma Mix
- PCR grade Water
- Positive Control (PC)
- Internal Control (IC)

- incubate
- vortex
- centrifuge
- add
- data reading

n = number of required reactions
NTC = No Template Control
* Not necessary, if it has already been added during extraction.
**Assay control:** Prepare a 1:10 dilution of the reconstituted IC.

This procedure overview is not a substitute for the detailed manual.

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



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