

Cyclus® RT-qPCR Mycoplasma

RT-qPCR for
EP 2.6.7-Compliant
Mycoplasma Detection
in ATMPs and
Biopharmaceuticals



Benefits

- Time-to-result: Only a few hours
- EP 2.6.7 (12.2)-aligned sensitivity: ≤ 10 CFU/mL & < 100 GC/mL
- RT-qPCR detecting DNA and RNA for maximum sensitivity
- Internal control added before extraction for full workflow inhibition monitoring
- Viable mycoplasma used for primary validation; This supports product-specific validation with Cyclus® 10 CFU (inactivated CFU) and Cyclus® 100 GC Standards
- Combined with Cyclus® Bead Extraction kit enables (high throughput) manual or automated workflows
- Robust lyophilized reagents – No need for dry ice shipment or frozen storage
- TaqMan® probe chemistry with FAM™ and HEX™/VIC™ channels for high specificity and clear signal separation

Product Information

Cyclus® RT-qPCR Mycoplasma is a RT-qPCR assay for rapid and sensitive detection of Mycoplasma, Acholeplasma and Spiroplasma in cell cultures, biopharmaceutical products and advanced therapy medicinal products (ATMPs).

The assay is aligned with the revised European Pharmacopoeia chapter 2.6.7 "Mycoplasmas" (12.2) and supports compliance with JP 18 G3 as well as USP <63> and USP <77> (draft).

In combination with the Cyclus® Bead Extraction kit and Cyclus® 10 CFU and 100 GC Standards, it provides a robust, pharmacopoeia-compliant solution for routine QC and method validation.

Applications

Cyclus® RT-qPCR Mycoplasma is intended for:

- In-process and release testing of cell culture-based biopharmaceuticals
- QC of viral vectors, vaccines, recombinant proteins and ATMPs
- Mycoplasma testing of master/working cell banks and production cultures
- Product-specific validation of NAT-based mycoplasma methods according to EP 2.6.7, JP 18 G3 and USP

Fast Workflow

- Sample Prep: Use 250 µl sample for nucleic acid extraction with Cyclus® Bead Extraction
- PCR Setup: RT-qPCR setup with lyophilized Mycoplasma Mix, Internal Control and Positive Control
- PCR Run & Analysis: Result interpretation based on FAM™ (target) and HEX™/VIC™ (IC) signals and run controls

Assay Principle, Probes and Channels

- Assay type: Reverse transcription quantitative PCR (RT-qPCR) with TaqMan® probes
- Targets: Mycoplasma, Acholeplasma and Spiroplasma (Mollicutes)
- Detection channels:
 - FAM™ (or equivalent): Mycoplasma target
 - HEX™/VIC™ (or equivalent): Internal Control
- Required run controls:
 - No Template Control (NTC)
 - Negative Extraction Control (NEC)
 - Internal Control (IC)
 - External Positive Control (EPC, not included; Cyclus® 10 CFU or Cyclus® 100 GC Standards recommended)

Technical Information

Performance

- Sensitivity: ≤ 10 CFU/mL and < 100 GC/mL (depending on species and matrix)
- Specificity: detects >130 Mollicutes species; no detection of eukaryotic DNA; specificity evaluated against related non-mycoplasmal bacteria according to EP 2.6.7 (12.2)
- Robustness: High cell backgrounds were tested during primary validation

Kit Components

(Cyclus® RT-qPCR Mycoplasma, SMB95-6002)

Label of tube	Cap color of tube	SMB95-6002 (25 reactions)
Mycoplasma Mix	red	1 × lyophilized
Internal Control	yellow	1 × lyophilized
Positive Control	green	1 × lyophilized
PCR grade Water	white	2 × 2 mL

Critical kit components are lyophilized, therefore less temperature sensitive to ensure highest performance and stability. The kit is stored at +2 °C to +8 °C until use. After rehydration, the Mycoplasma Mix storage at < -18 °C is necessary and only possible when used within 30 days. After rehydration, the Internal Control and Positive Control can be stored at < -18 °C until their specified shelf life printed on the package label. The kit is manufactured under certified quality management, with lot-specific QA certificates available via the MySartorius Portal:

<https://my.sartorius.com>

User-Supplied Equipment and Material

- For nucleic acid extraction Cyclus® Bead Extraction from Sartorius is needed (SMB95-6000) which can be run manually using a magnetic stand or automated on a KingFisherFlex™ instrument
- 1.5 mL reaction tubes (DNase-, RNase-free)
- Microcentrifuge for 1.5 mL reaction tubes
- Suitable pipettes for pipetting different volumes, 10, 100 and 1,000 µL (offered by Sartorius)
- Compatible pipette filter tips, DNase-, RNase-free (offered by Sartorius)
- PCR reaction tubes, strips or plates and caps, recommended by the manufacturer of the respective thermal cycler
- qPCR device with filter sets for detecting the fluorescent dyes FAM™ and HEX™ (or alternatively filter sets for detecting VIC® or JOE™)
- For External Positive Control and EP 2.6.7 (12.2) compliant testing Cyclus® 100 GC or Cyclus® 10 CFU Mycoplasma Standards available for all EP-/JP-/USP-listed mycoplasma species (see Related Products section)

Ordering Information

Mycoplasma Kits

SMB95-6002	Cyclus® RT-qPCR Mycoplasma	25 reactions
------------	----------------------------	--------------

Related Products

Nucleic Acid Extraction Kit

SMB95-6000	Cyclus® Bead Extraction	50 extractions
SMB95-6003	Cyclus® Bead Extraction Lysis Buffer	27.5 mL

Cyclus® 100 GC, 3 vials

SMB95-3001	Cyclus® 100 GC <i>Mycoplasma arginini</i>	3 vials
SMB95-3002	Cyclus® 100 GC <i>Mycoplasma orale</i>	3 vials
SMB95-3003	Cyclus® 100 GC <i>Mycoplasma gallisepticum</i>	3 vials
SMB95-3004	Cyclus® 100 GC <i>Mycoplasma pneumoniae</i>	3 vials
SMB95-3005	Cyclus® 100 GC <i>Mycoplasma synoviae</i>	3 vials
SMB95-3006	Cyclus® 100 GC <i>Mycoplasma fermentans</i>	3 vials
SMB95-3007	Cyclus® 100 GC <i>Mycoplasma hyorhinis</i>	3 vials
SMB95-3008	Cyclus® 100 GC <i>Acholeplasma laidlawii</i>	3 vials
SMB95-3009	Cyclus® 100 GC <i>Spiroplasma citri</i>	3 vials
SMB95-3010	Cyclus® 100 GC <i>Mycoplasma salivarium</i>	3 vials

Cyclus® 10 CFU, 3 vials

SMB95-3011	Cyclus® 10 CFU <i>Mycoplasma arginini</i>	3 vials
SMB95-3012	Cyclus® 10 CFU <i>Mycoplasma orale</i>	3 vials
SMB95-3013	Cyclus® 10 CFU <i>Mycoplasma gallisepticum</i>	3 vials
SMB95-3014	Cyclus® 10 CFU <i>Mycoplasma pneumoniae</i>	3 vials
SMB95-3015	Cyclus® 10 CFU <i>Mycoplasma synoviae</i>	3 vials
SMB95-3016	Cyclus® 10 CFU <i>Mycoplasma fermentans</i>	3 vials
SMB95-3017	Cyclus® 10 CFU <i>Mycoplasma hyorhinis</i>	3 vials
SMB95-3018	Cyclus® 10 CFU <i>Acholeplasma laidlawii</i>	3 vials
SMB95-3019	Cyclus® 10 CFU <i>Spiroplasma citri</i>	3 vials
SMB95-3020	Cyclus® 10 CFU <i>Mycoplasma salivarium</i>	3 vials

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

For technical support, validation guidance or assay design questions, please contact:

PCR@Sartorius.com

Germany

Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Straße 20
37079 Göttingen
Phone +49 551 308 0

USA

Sartorius Corporation
3874 Research Park Drive
Ann Arbor, MI 48108
Phone +1 734 769 1600

 **For further information, visit**
[sartorius.com](https://www.sartorius.com)