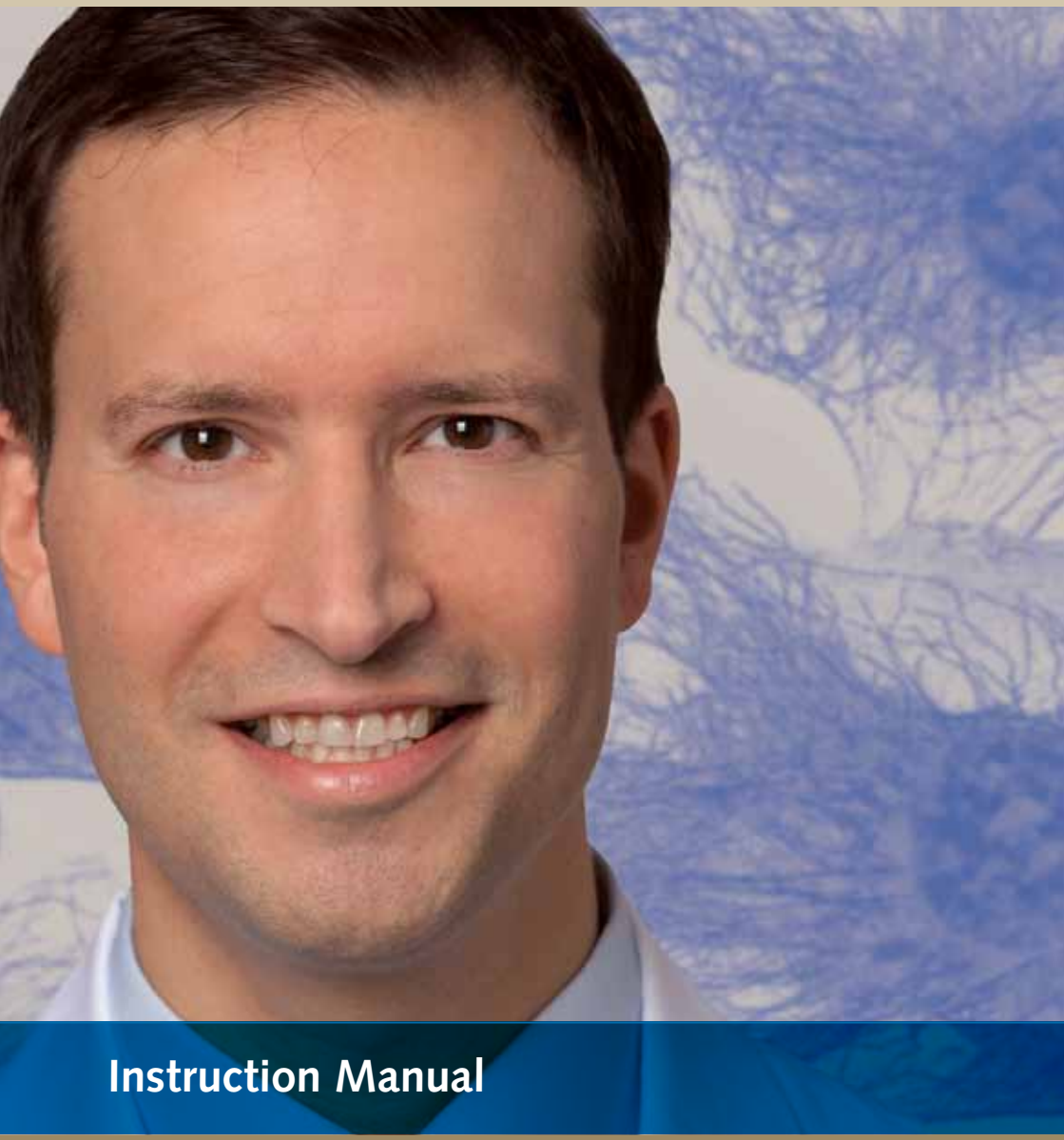
PCR Mycoplasma Test Kit I/RT



Instruction Manual

PromoKine

Contents

Description

The contamination of cell cultures with mycoplasmas is a common and serious issue in biological research and in the pharmaceutical industry, which can lead to non-reproducible results and significant productivity losses. It is a requirement for all cell culture laboratories to avoid these problems by routine diagnostic testing and identifying the source for contamination with mycoplasmas in order to fulfill the quality control standards. The Mycoplasma Test Kit I/RT for quantitative PCR is a high sensitive and fast test system for detection of mycoplasma contamination in cell cultures and other cell culture derived biologicals. The test is specific to a broad range of Mycoplasma species and exhibits no cross reactivity to phylogenetically related bacteria or eukaryotic DNA.

The kit contains all reagents required for real-time polymerase chain reaction: nucleotides, primer and probes, reaction buffer, polymerase and controls. After rehydration of the components, the PCR Master Mix is easily prepared by just adding the Inhibition Control DNA to the Primer&Probes.

The PCR is initiated with a 2 minute high-temperature step to melt all nucleic acids and to activate the polymerase. In a successful PCR, the **presence of mycoplasmas will be indicated by a signal in the FAM channel**. The **Inhibition Control DNA** is used as an internal amplification control that will be detected in the **HEX channel (kit variant C)**. Thus, false-negative results, due to inhibition of the reaction by the sample matrix, can be excluded individually for each sample. The kit contains the nucleotide dUTP instead of dTTP and is therefore suitable for UNG pretreatment to prevent carry over between PCRs.

Note: Your Real-Time PCR instrument requires the channels/filters for FAM and HEX. In case you are not sure whether your cycler is compatible with FAM/HEX, please contact your instrument supplier.

Materials Included / Packing List

Kit Variant C

- **Primer&Probes**, containing mycoplasma-specific primer set; nucleotides dATP, dCTP, dGTP and dUTP; Taq Polymerase; lyophylized; for 25 or 50 reactions
- **Inhibition Control DNA**, containing plasmid DNA, lyophylized, non-infectious; for at least 25 or 50 reactions
- **Positive Control DNA**, containing plasmid DNA, lyophylized, non-infectious; for at least 25 or 50 reactions
- **Rehydration/Reaction Buffer**
- **DNA-free Water**
- **Instruction manual**

Specificity

The primer set amplifies and the probe detects a DNA sequence within the highly conserved 23S rRNA operon coding region in the Mycoplasma genome. The detection spectrum includes most mycoplasma species identified as cell culture contaminants and listed according European Pharmacoipea, e.g. *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma hyorhinis*, *Mycoplasma argininii, Mycoplasma salivarium, Mycoplasma hominis, Mycoplasma genitalium, Mycoplasma synoviae, Mycoplasma pulmonis, etc.* but also *Mycoplasma pneumoniae* and *Acholeplasma laidlawii*. The target probe emits fluorescent light at 520 nm. Please see our website for a complete list of detectable species.

Cross-detection of bacteria with close phylogenetic relation to Mycoplasma is not monitored. The „European Pharmacopoeia" recommends to check for unspecific detection of Clostridium, Lactobacilllus, and Streptococcus. None of the following species is detected with the PCR Mycoplasma Test Kit I/RT kit: Clostridium acetobutylicum, *Lactobacillus acidophilus* and *Streptococcus pneumoniae*. Furthermore there is no positive signal with human, murine or other bacterial DNA (e.g.: *Legionella sp.*, *Chlamydophila sp.*, *Bordetella sp.*) as template.

Storage Conditions and Shelf Life

Kit components are stable during shipping at +2°C to +8°C. Upon receipt, store at 4°C until use.

After reconstitution of lyophilized components, **avoid repeated freezing and thawing** and freeze aliquots for future use at < -18°C. When following the recommendations, the kit components are stable until the expiration date indicated on the label. Please protect the Primer&Probes from light.

Preparation of Sample Material

DNA extraction with a commercially available DNA extraction kit is always advisable when preparing the samples in order to remove inhibitors of the PCR safely and to concentrate the mycoplasma DNA at greater sample volumes. Also dyes that can influence fluorescence measurement can be removed. The obtained DNA extract can be used directly for the Real-Time qPCR test. Samples should be derived from cultures which are at 90-100 % confluence.
When testing samples without DNA extraction, PCR inhibiting substances may accumulate in the medium of older cultures. Only cell culture supernatant should be applied to test for mycoplasma. Cell pellets should not be tested, since debris will interfere with the PCR reaction. It is recommended not to use > 2 µl sample volume for direct testing of cell culture supernatants. With average titers at 10^6 and a maximum titer at 10^8 you will find sufficient mycoplasma in the supernatant to guarantee a sensitive PCR. If there is no amplification of the inhibition control in mycoplasma negative samples, a DNA extraction is necessary to exclude false negative results. Penicillin or streptomycin in the culture media do not inhibit mycoplasmas or affect test sensitivity.
However, other materials that can be tested are Fetal Calf Serum, vaccines, and paraffin-embedded samples following DNA extraction. To avoid false positive results, we recommend the use of deionized, DNA-free H_2O, aerosol-preventive filter tips and gloves.

The following method is recommended:

1. Transfer or 500 µl sample material, e.g. cell culture supernatant, into a sterile DNA-free reaction tube

2. Incubate the sample supernatant at 95°C for 5 minutes
3. Centrifuge the supernatant (10,000 x g, 5 seconds) to remove cellular debris
4. Use 2 µl of the extract as template

Procedure

Rehydration of the Reagents

1. Centrifuge the Lyophilized reagents for 5 seconds in a table centrifuge
2. Add 390 µl of Reaction Buffer to the Primer&Probes
3. add 300 µl deionized, DNA free water to the Inhibition Control DNA
4. add 300 µl deionized, DNA free water to the Positive Control DNA
5. incubate for 5 minutes at room temperature
6. vortex and spin down for 5 seconds

The PCR Master Mix

Prepare one master mix with water, Primer&Probes, Inhibition Control and Polymerase. Aliquot 16-23 µl* (dependent on the sample volume) of the mixes into the PCR tubes. Add the sample, Positive Control DNA or - in case of the negative control - add water to the master mix.

Pipetting scheme for one reaction:	
DNA-free water	8.0 µl*
Primer&Probes	14.0 µl
Inhibition Control DNA	1.0 µl
Total volume	23.0 µl
Add sample	2.0 µl*

* 2-10 µl sample volume can be used; please lower the volume of water corresponding to the increased sample volume (add water to sample to obtain a final volume of 10 µl; e.g., 5 µl sample: add 5 µl water).

Programming and Data Recording of Different qPCR Devices

1. For the LightCycler® 2.0

Program 1: Preincubation	
Cycles	1
Analysis Mode	None
Temperature Targets [°C]	*Segment 1*
Target Temperature [°C]	95
Incubation time [min]	2:00
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2: Amplification	
Cycles	45
Analysis Mode	Quantification

Temperature Targets [°C]	*Segment 1*	*Segment 2*	*Segment 3*
Target Temperature [°C]	95	55	60
Incubation time [s]	15	30	45
Temperature Transition Rate [°C/s]	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0.0	0.0	0.0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	None	Single

Note: Segment 3 is essential for the performance of the kit. Do not skip or modify this step.

Program 3: Cooling

Cycles	1
Analysis Mode	None
Temperature Targets [°C]	*Segment 1*
Target Temperature [°C]	40
Incubation time [s]	30
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Note: Please adjust the „seek temperature“ to at least 90°C.

Detector Settings:

LightCycler® 2.0

Target Probe:	Channel 1 (530 nm)
Inhibition Control:	Channel 2 (555 nm)

2. For ABI Prism® and Stratagene Cyclers (also recommended for other thermal block cyclers such as LightCycler® 480)

Detector Settings:

Target Probe:	Reporter – FAM
Internal Control:	Reporter – HEX
	Quencher – none

Note: The ROX Reference needs to be disabled (for ABI Prism® Cyclers).

Programming scheme for ABI Prism® Cyclers shown for ABI7500
Initial Polymerase activation:

Initial Polymerase activation: 95°C; 180 seconds
Cycling 45 x

95°C: 30 sec	Denaturation
55°C: 30 sec	Annealing
60°C: 45 sec	Amplification and data collection

3. For other real-time PCR Instruments, e.g. SmartCycler® II

Detector Settings:

Target Probe:	Reporter – FAM
Internal Control:	HEX
	Quencher – none

Note: The ROX Reference needs to be disabled.

Programming scheme:
Initial Polymerase activation: 95°C; 180 seconds
Cycling 45x

95°C for 15 sec	optics off
55°C for 30 sec	optics off
60°C for 45 sec	optics on

Test Evaluation

A mycoplasma contamination is indicated by an increasing fluorescence signal in the "FAM-Channel" during PCR. In reactions containing the Inhibition Control DNA a signal in the "HEX-Channel" will be generated. However, the inhibition control reaction may be outcompeted by a strong target reaction.

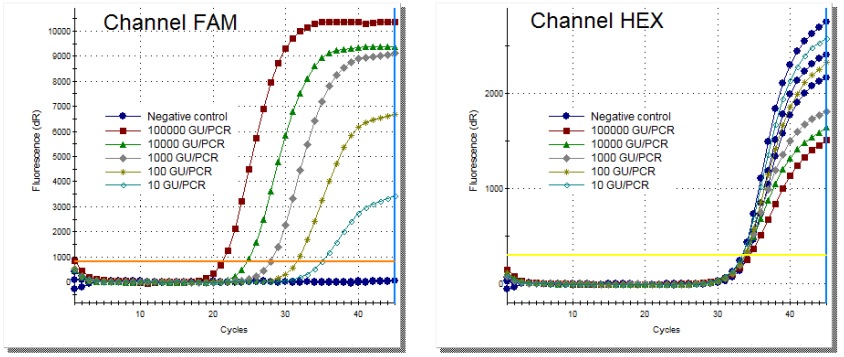


Figure 1: Amplification plots of a dilution series of genomic DNA preparations of Mycoplasma fermentans. As negative controls, the template DNA was replaced with PCR-grade water. The reactions were performed on an Mx3005P (Agilent Technologies). A) Detection of target amplification in FAM channel; B) monitoring of inhibition control amplification in HEX channel.

Troubleshooting

No amplification of Inhibition Control Spike may be due to the following reasons:

- activity of polymerase is insufficient
- sample DNA inhibits PCR reaction
- Primer/Probe/Nucleotide Mix tubes and/or Inhibition Control Spike have not been spun down before rehydration
- programming mistake
- pipetting mistake

Before repeating PCR please check thermocycler protocol and pipetting scheme. When using polymerases in addition to the *Taq* DNA Polymerase provided with the kit we cannot guarantee optimal results.

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Promocell 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.

Precautions

MSDS is available on our website or upon request.

Trademarks

LightCycler is a registered trademark of a member of the Roche Group. ABI Prism is a registrated trademark of Applera Corporation or its subsidiaries in the US and certain other countries. SmartCycler is a registered trademark of Cepheid.

Related Products

Catalog Number	Product Name	Product Description	Unit Size
Mycoplasma Elimination			
PK-CC03-036-1B	BIOMYC-1	antibiotic solution, 100x	100 ml
PK-CC03-036-1C	BIOMYC-1	antibiotic solution, 100x	20 ml
PK-CC03-036-1D	BIOMYC-1	antibiotic solution, 100x	10 ml
PK-CC03-037-1B	BIOMYC-2	antibiotic solution, 100x	100 ml
PK-CC03-037-1C	BIOMYC-2	antibiotic solution, 100x	20 ml
PK-CC03-037-1D	BIOMYC-2	antibiotic solution, 100x	10 ml
PK-CC03-038-1B	BIOMYC-3	antibiotic solution, 100x	100 ml
PK-CC03-038-1C	BIOMYC-3	antibiotic solution, 100x	20 ml
PK-CC03-038-1D	BIOMYC-3	antibiotic solution, 100x	10 ml
PK-CC91-4003	Mycoplasma-EX	non-antibiotic/antibiotic solution	3 treatments
Disinfection of Surfaces & Maintenance of Water Quality			
PK-CC01-867-1B	Aquaguard-1	For CO_2 incubator water (tray)	100 ml
PK-CC01-867-3B	Aquaguard-1	For CO_2 incubator water (tray)	3 x 100 ml
PK-CC01-867-10B	Aquaguard-1	For CO_2 incubator water (tray)	10 x 100 ml
PK-CC01-916-500	Aquaguard-2	For ordinary water baths	500 ml
PK-CC91-5051	Mycoplasma-ExS Spray	Surface Disinfection (Lab Equipment)	500 ml
PK-CC91-5051-10	Mycoplasma-ExS Spray	Surface Disinfection (Lab Equipment)	10 x 500 ml
PK-CC91-5052	PromoCidal Spray	Surface Disinfection (Lab Equipment)	500 ml
PK-CC91-5052-5L	PromoCidal Spray	Surface Disinfection (Lab Equipment)	5 L
PK-CC91-5052-W100	PromoCidal Wipes	Surface Disinfection (Lab Equipment)	100 pieces
PK-CC91-5053	Spore-EX Spray	Surface Disinfection (Lab Equipment)	500 ml
PK-CC91-5053-W120	Spore-EX Wipes	Surface Disinfection (Lab Equipment)	120 pieces
PK-CC91-5061	Mycoplasma Disinfection Kit I	Consists of Mycoplasma-ExS Spray, Aquaguard-1 and Aquaguard-2	1 Kit
PK-CC91-5071	Mycoplasma Disinfection Kit II	Consists of PromoCidal Spray, Aquaguard-1 and Aquaguard-2	1 Kit
Mycoplasma Detection			
Catalog Number	**Product Name**	**Product Description**	**Unit Size**
PK-CA91-1024	PCR Mycoplasma Test Kit I/C	For conventional PCR	24 assays
PK-CA91-1048	PCR Mycoplasma Test Kit I/C	For conventional PCR	48 assays
PK-CA91-1096	PCR Mycoplasma Test Kit I/C	For conventional PCR	96 assays
PK-CA20-700-20	PCR Mycoplasma Test Kit II	For conventional PCR	20 assays

Mycoplasma Detection			
PK-CA91-0112	Mycoplasma orale DNA Standard (for qPCR)	Defined quantification standard for the Real-Time PCR Mycoplasma Test Kits (1.000.000 genomes/µl)	100 µl
PK-CA91-0117	Mycoplasma fermentans DNA Standard (for qPCR)	Defined quantification standard for the Real-Time PCR Mycoplasma Test Kits (1.000.000 genomes/µl)	100 µl
Bacteria Detection			
PK-CA91-2024	PCR Bacteria Test Kit	For conventional PCR	24 assays
PK-CA91-2048	PCR Bacteria Test Kit	For conventional PCR	48 assays

Ordering Information

Product Name	Size	Catalog Number
PCR Mycoplasma Test Kit I/RT, Variant C	25 tests	PK-CA91-3025C
PCR Mycoplasma Test Kit I/RT, Variant C	50 tests	PK-CA91-3050C

For in vitro research use only.
Not for diagnostic or therapeutic procedures.

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