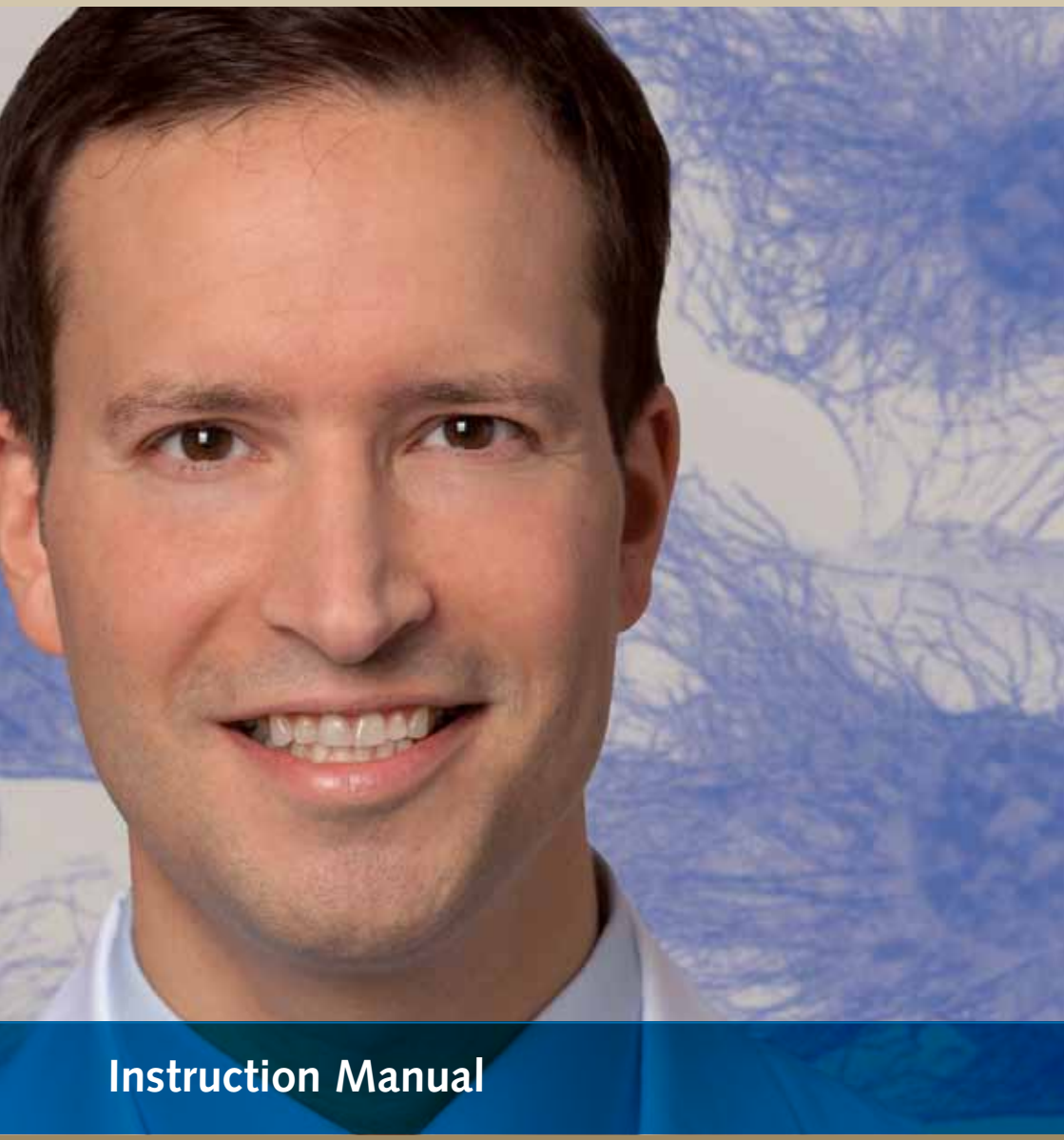
PCR Mycoplasma Test Kit I/C



Instruction Manual

PromoKine

Contents

Description

The test reaction tubes contain all Master Mix reagents required for conventional Polymerase chain reactions: internal control DNA, nucleotides, primer, Hot-Start Taq DNA Polymerase and an inert gel-loading dye for agarose gel electrophoresis. Thus, only the *PCR Rehydration Buffer* needs to be added. The reagents of the reaction tubes are dissolved in the *Rehydration Buffer*. PCR is initiated with a 2 minute high-temperature step to melt all nucleic acids and to activate the *Taq DNA Polymerase*. After completion of PCR, a portion of the reaction can be loaded directly onto an agarose gel for characterization. There is no need to add gel loading buffer or dye. The kit contains a *Positive Control DNA* to assure reproducibility.

Materials Included / Packing List

- Instruction Manual.
- 24 (3 x 8, PK-CA91-1024) / 48 (6 x 8, PK-CA91-1048) / 96 (12 x 8, PK-CA91-1096) **Test reaction tubes** with lyophylized Master Mix containing: primer set; nucleotides dATP, dCTP, dGTP and dUTP; Hot-Start Taq DNA Polymerase and internal control DNA.
- 8 (1 x 8, PK-CA91-1024) / 16 (2 x 8, PK-CA91-1048) / 24 (3 x 8, PK-CA91-1096) **Positive Control reaction tubes** with lyophylized Master Mix containing: primer set; nucleotides dATP, dCTP, dGTP and dUTP; Hot-Start Taq DNA Polymerase and DNA-fragments of the *Mycoplasma orale* genome (prepared by PCR, non-infectious) as well as internal control DNA.
- Rehydration Buffer, 1.6 ml (PK-CA91-1024) / 1.6 ml (PK-CA91-1048) / 3.2 ml (2 x 1.6 ml, PK-CA91-1096).
- PCR tube caps.

Note: Do not mix different lot numbers of any kit component!

Specificity

The primer set amplifies a DNA sequence within the highly conserved 16S rRNA operon coding region in the *Mycoplasma* genome. The detection spectrum includes *Mycoplasma orale*, *Mycoplasma fermentans*, *Mycoplasma hyorhinis*, *Mycoplasma arginini*, *Mycoplasma salivarium*, *Mycoplasma hominis*, *Acholeplasma laidlawii* and many other species. This kit does not detect the clinically relevant species *Mycoplasma pneumoniae* and *Ureaplasma urealyticum* which are not known as cell culture contaminants. Eukaryotic DNA is not amplified. A successfull PCR reaction is indicated by a distinct 479 bp band derived from the internal control DNA on the agarose gel.

The kit was thoroughly validated with different sample materials (chondrocytes, serum, cell culture supernatant, etc.) and different mycoplasma species most commonly detected in cell cultures.

Storage Conditions

The kit is shipped at room temperature. Upon receipt, the kit components can be stored at 4-8°C. Store rehydrated components at -20°C.
Important: The strips should be stored in a dry environment. Be careful to avoid mechanical damage of the sealing foil before using the vials, when handling/separating tubes. Please note that a damaged or leaking sealing foil will not protect the lyophilisate from humidity resulting in “sticky pellets”.

Shelf Life

By following the recommendations, the kit is stable until the expiration date indicated on the label.

Procedure

Preparation of Sample Material

Cell lines should be pre-cultured in the absence of mycoplasma active antibiotics for 2 passages to maximize test sensitivity.

For later PCR analysis stable templates are prepared by boiling the supernatant of cell cultures or other biologicals for 10 minutes as follows:

Transfer 100 µl of supernatant from the test culture to a sterile microcentrifuge tube. The lid should be tightly sealed to prevent opening during heating.

1. Heat the sample supernatant at 95°C for 10 minutes.
2. Briefly centrifuge (5 seconds) the sample supernatant to pellet cellular debris before adding to the PCR mixture.
3. The templates are stable at 2°C to 8 °C up to five days and at -20°C for long-term storage.

For highest sensitivity mycoplasma can be concentrated by centrifugation as follows:

1. Transfer 1 ml of cell culture supernatant to a microcentrifuge tube.
2. Spin at 500 x g for 5 minutes to pellet cellular debris.
3. Transfer the supernatant to a fresh tube.
4. Centrifuge at a minimum of 14,000 x g for 15 minutes.
5. Decant the supernatant and resuspend the mycoplasma pellet in 100 µl of fresh cell culture medium or DNA free water.
6. Prepared samples can be used either directly or boiled as described above for long term storage.

Note: Samples should be derived from cultures that are at 90-100 % confluence. PCR inhibiting substances may accumulate in the medium of older cultures. For a sample from an older culture or samples containing high amounts of serum (e.g. >10% FCS), proteins or DMSO (e.g. cryogenic samples), a DNA extraction is strictly recommended prior to testing.

Reaction Setup

Total volume per reaction is 25 µl. Please perform a positive control reaction (step 4) in each run.

1. **Rehydration of test reactions with the Rehydration Buffer**

   Take a strip of *Test Reaction Tubes* out of the bag and cut off the number of tubes needed. Put back the remaining tubes and close the zip lock bag. Peel off the protection film from the tubes. Rehydrate the lyophylized components of the tubes by adding 23 µl *Rehydration Buffer* to each PCR tube.
   Note: For optimal PCR, tubes need to be in close contact with the heating element. Thus, ensure that the tubes fit well into the wells of the heating element. If complete tube strips or attached tubes do not fit properly into the wells without tension, you will have to cut apart the tubes.

2. **Add the sample**

   Add 2 µl **fresh cell culture medium or DNA Elution Buffer*** to the negative control and 2 µl of sample to the test reactions (23 µl + 2 µl). Close the tubes with the caps included in the kit.

3. **Rehydration of Positive Control reactions with Rehydration Buffer**

   Take a strip of Positive Control tubes out of the bag and cut of the number of tubes needed. Put back the remaining tubes and close the zip lock bag. Peel of the protection film from the tubes. To rehydrate the lyophylized components of the tubes add 23 µl *Rehydration Buffer* to the Positive Control tube and add 2 µl of **DNA-free water**. Close the tubes with the caps included in the kit.

4. **Incubation**

   Mix the content thoroughly by flickering the tube. Do not vortex! Allow the lyophilized components to dissolve by incubating for 5 minutes at room temperature. Proceed to thermal cycling.

Table 1: Pipetting scheme

	Test Sample	**Positive Control**	**Negative Control**
Rehydration Buffer	23 µl	23 µl	23 µl
Sample	2 µl		
DNA-free water		2 µl	
Fresh Cell Culture Medium*			2 µl

***Note:** In case you had to extract your sample DNA using a DNA extraction kit or purification column, please use 2 µl of the respective elution buffer to perform the negative control.

PCR

Place tubes in thermal cycler. Enter and run the following program.

1 cycle	95°C for 2 minutes
40 cycles	94°C for 30 seconds
	55°C for 30 seconds
	72°C for 40 seconds
	cool down to 4-8°C

Note: The 2 minute-step at 95°C is required to activate the *Taq DNA Polymerase*.

Agarose Gel Run

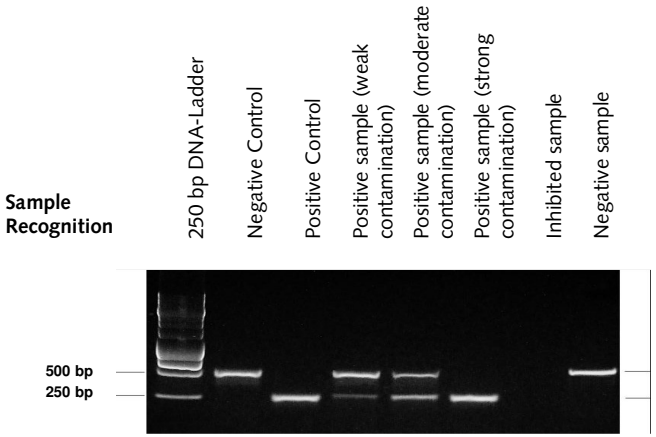
To ensure the thorough mixing of the reaction components, please vortex the reaction tube gently prior to agarose gel electrophoresis. A centrifugation step might be necessary to spin the complete reaction solution down to the bottom of the tube. You may then proceed with the agarose gel run.

- Use a 1.5% standard agarose gel (maximal 5 mm thick!) with a 5 mm-comb.
- Load 8 µl of each PCR reaction per lane. It is not recommended to add extra loading buffer.
- Stop electrophoresis after a 2-3-cm run distance (depending on the electrophoresis chamber used, e.g. run for 25 minutes at 100 V).

Gel Evaluation

- A **mycoplasma-positive sample** shows a distinct band at **265-278 bp**.
- The **Negative Control** and **negative samples** show the internal control band at **479 bp**.
- The **Positive Control** shows a band at **270 bp** and - depending on the setup - an additional band of the internal control at 479 bp (see next point).
- The **internal control DNA** shows a distinct **479 bp** band and should appear in every lane indicating a successfully performed PCR. This band may fade with increased amount of amplicons formed due to mycoplasma DNA amounts of > 10^4 copies/ml. Thus, the internal control may also not be visible in the positive control reaction.
- No amplification reactions in a sample but in the Negative as well as in the Positive Controls indicates PCR inhibition due to the sample DNA. If the PCR of a sample is inhibited, PCR inhibitors can easily be removed from the sample DNA by performing a DNA extraction/clean-up using a commercially available kit.

Table 2: Typical Agarose Gel Picture



Troubleshooting

- Before repeating a negative and a positive control, please check thermocycler protocol and pipetting scheme.
- The PCR Mycoplasma Test Kit has been designed for high sensitivity and is therefore prone to nonspecific annealing. Bands of various lengths that are less intensive can be produced, but do not indicate positive results. Possible primer self-annealing produces another band of 80-90 bp in length, but also does not affect the precision or results of the test.
- Inhibition of the PCR reaction has been observed with samples containing a higher serum concentrations (>10% v/v), cultures grown to 100% confluence or even longer showing a yellowish color of the medium and cell debris.

Frequently Asked Questions

What kind of samples can be tested?

The test kit can be used for suspension or adherent cells in culture, primary cells, virus stocks and other biological. Culture supernatants are the preferred sample material. Other materials, like cryo stocks, vaccines, and paraffin-embedded samples require a suitable DNA purification step.

What sample volume is required?

The kit requires 2 µl sample volume for each reaction.

What is the detection limit?

The detection limit is approx. 15 fg of mycoplasma DNA that corresponds to 15 mycoplasma per sample volume.

What is the best moment for sampling?

The cells should be cultivated to 90% confluence to guarantee a maximal density of mycoplasmas in the sample volume.

Does the kit detect all relevant mycoplasma species?

Species specificity includes the following: *A. laidlawii*, *M. agalactiae*, *M. arginini*, *M. arthritidis*, *M. bovis M. cloacale*, *M. falconis*, *M. faucium*, *M. fermentans*, *M. hominis*, *M. hyorhinis*, *M. hyosynoviae*, *M. opalescens*, *M. orale*, *M. primatum*, *M. pulmonis*, *M. salivarium*, *M. spermatophilum*, *and M. timone*. This kit is not suitable for detection of *M. pneumoniae*, *U. urealyticum*, or other clinically associated species.

Does the kit allow a semi-quantitative interpretation?

A band at ~270 bp depicts a positive result regardless of its intensity. A band of weak intensity represents a low contamination level, whereas a band of greater intensity represents a high contamination level.

What about unspecific bands?

Unspecific band on the gel are extremely rare. Only an amplicon at ~270 bp represents a positive result. All other bands do not correspond to the mycoplasma genome. Due to the extreme diversity no forecast for particular sample material is possible.

Do I need additional reagents?

The kit contains all required reagents. For positive control samples 2 µl of DNA-free, UV-irradiated water is recommended. However, freshly distilled water, gamma-irradiated water, or any other method for obtaining **DNA-free water** is also acceptable.

What controls should be performed?

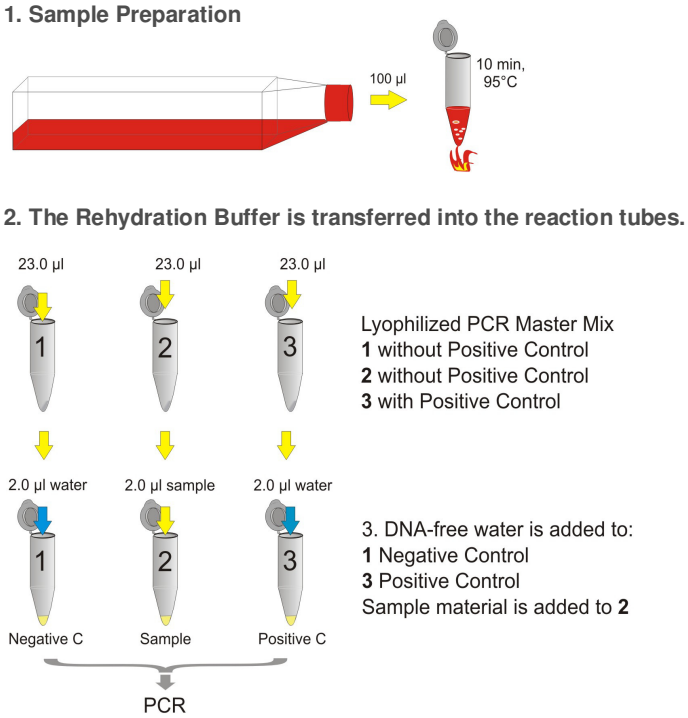
It is highly recommended that both **positive and negative control reactions** be performed for each test series. These controls are to insure assay conditions, as well as biological positive controls. A negative control reaction, in which the sample volume is replaced by sterile, DNA-free water – or better – **fresh cell culture medium or DNA Elution Buffer (if a DNA extraction has been done)**, should also be performed.

Can I omit the internal control?

The internal control is an absolute requirement to ensure the quality of the PCR. Due to the sample matrix are mistakes during the pipetting a PCR can fail. Without the internal control no reliable interpretation is possible. The internal control has no influence of the sensitivity of the test system and is therefore directly integrated in the reagent mix.

For more FAQs and trouble-shooting please visit our website (see "Knowledge Base").

Schematic Presentation of the Procedure



Related Products

Catalog Number	Product Name	Product Description	Unit Size
Mycoplasma Elimination			
PK-CC91-4003	Mycoplasma-EX	non-antibiotic + antibiotic solution	3 treatments
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
Surface Disinfection & Water Stabilizers			
PK-CC01-867-1B	Aquaguard-1	CO_2 incubator water bath disinfection	100 ml
PK-CC01-867-3B	Aquaguard-1	CO_2 incubator water bath disinfection	3 x 100 ml
PK-CC01-867-10B	Aquaguard-1	CO_2 incubator water bath disinfection	10 x 100 ml
PK-CC01-916-500*	Aquaguard-2	Ordinary water bath disinfection	500 ml
PK-CC91-5051*	Mycoplasma-ExS Spray	Lab Surface Disinfection	500 ml
PK-CC91-5051-10*	Mycoplasma-ExS Spray	Lab Surface Disinfection	10 x 500 ml
PK-CC91-5052*	Promocidal Spray	Lab Surface Disinfection	500 ml
PK-CC91-5052-5L*	PromoCidal Spray	Lab Surface Disinfection	5 L
PK-CC91-5053*	Spore-EX Spray	Lab Surface Disinfection	750 ml
PK-CC91-5052-W120*	Spore-EX Wipes	Lab Surface Disinfection	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

*** not available in the US.**

Catalog Number	Product Name	Product Description	Unit Size
Mycoplasma Detection			
PK-CA20-700-20	PCR Mycoplasma Test Kit II	For conventional PCR	20 assays
PK-CA91-3025C	PCR Mycoplasma Test Kit I/RT, Variant C	For Real Time qPCR	25 assays
PK-CA91-3050C	PCR Mycoplasma Test Kit I/RT, Variant C	For Real Time qPCR	50 assays
PK-CA91-0112	Mycoplasma orale DNA Standard (for qPCR)	Defined quantification standard for the Real-Time PCR Mycoplasma Test Kits	100 µl
PK-CA91-0117	Mycoplasma fermentans DNA Standard (for qPCR)	Defined quantification standard for the Real-Time PCR Mycoplasma Test Kits	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/C	24 tests	PK-CA91-1024
PCR Mycoplasma Test Kit I/C	48 tests	PK-CA91-1048
PCR Mycoplasma Test Kit I/C	96 tests	PK-CA91-1096

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

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