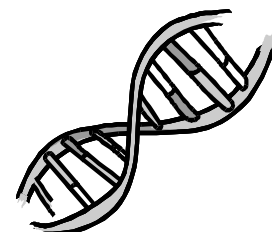



MutaPLATE[®]

Chlamydia pneumoniae

real time PCR Kit (TaqMan)



Qualitative assay for the specific detection of *Chlamydia pneumoniae* in clinical samples of the respiratory tract using *real time* PCR microplate systems (e.g. Applied Biosystems / Stratagene or Corbett Research).

REF KV1900496 



For research use only



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1. INTENDED USE

The **MutaPLATE® Chlamydia pneumoniae** *real time* PCR (TaqMan) kit is a qualitative screening assay for the detection of *Chlamydia pneumoniae* in clinical samples of the respiratory tract using microplate systems (e.g. Applied Biosystems, Stratagene, Corbett Research).

2. INTRODUCTION

Chlamydia pneumoniae is a widely distributed obligate intracellular pathogen causing upper and lower respiratory tract infections (f.e. bronchitis, sinusitis, etc.) and on the average 10% of all community-acquired pneumonias. *Chlamydia pneumoniae* is also suspected to cause arteriosclerosis causing thereby e.g. coronary artery disease. A seroprevalence of 40-70 % indicates that most people have been infected by this pathogen at least once in their lives.

3. PRINCIPLE OF THE TEST

The **MutaPLATE® Chlamydia pneumoniae** *real time* PCR (TaqMan) kit contains specific primers, TaqMan probes and additional material for the detection of *Chlamydia pneumoniae* in clinical respiratory tract samples by polymerase chain reaction (PCR). Target sequence for the detection is a region of the major outer membrane proteins (MOMP) of *Chlamydia pneumoniae*.

The amplification of possibly present *Chlamydia pneumoniae* DNA and the proof of specificity by hybridization of the amplicon specific TaqMan probe is done in one step. The TaqMan probe is labelled with a fluorescence dye on one end and a quencher molecule on the other end. In case of a *Chlamydia pneumoniae* specific amplicon, the emitted fluorescence signal is detected and quantified by the *real time* PCR microplate system`s optical unit (ABI: PRISM SDS-, Stratagene: MxPRO-, Corbett Research: Rotorgene 3000/ 6000-Software).

Chlamydia pneumoniae specific amplification is measured by FAM fluorescence.

To exclude a possible PCR inhibition, the amplification mix contains an internal control. The amplification of this internal control does not affect the *Chlamydia pneumoniae* detection and is measured by a probe`s VIC / HEX fluorescence.

4. KIT CONTENT

Each kit contains enough reagents to perform 96 tests. Each kit also contains a package insert.

Reference	Type of reagent	Presentation	Cap color
A1	Amplification Mix	3 x 1,5 ml	blue
A2	Positive control	1 x 0.5 ml	red
A3	Negative control	1 x 0.5 ml	green

5. TEST PERFORMANCE

Required materials - provided:

- PCR reagents
- Package insert

Required materials - not provided:

- ABI system or a comparable instrument (e.g. from Stratagene, Corbett Research)
- TC II reaction plate, 96 wells (Applied Biosystems) or comparable microtiter plates or reaction tubes to be used for optical detection within the two-parts holding frame (Applied Biosystems)
- Optical adhesive covers (Applied Biosystems) or comparable covers
- DNA extraction kit
- Pipets (0.5 µl – 200 µl) with sterile filter tips

6. STORAGE AND HANDLING

- All reagents (A1 to A3) should be stored at -20°C.
- All reagents can be used until the expiration date printed on the labels.
- Do not freeze and thaw the reagents A1, A2 and A3 several times. If used sporadically, prepare aliquots of the reagents.
- Cool all reagents during the working steps.

7. WARNINGS AND PRECAUTIONS

- For research use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials.
- This assay needs to be run according to GLP (Good Laboratory Practice).
- Do not use the kit after its expiration date.

AMPLIFICATION

The PCR technology is utmost sensitive. Thus, amplification of a single molecule generates millions of identical copies. These copies may evade through aerosols and sit on surfaces. In order to avoid contamination of samples with DNA which previously was amplified, it is important to physically strictly divide sample and reagent preparation units from sample amplification units. Set up two separate working areas:

- 1) Isolation of the DNA
- 2) Amplification/ detection of amplification products

Pipets, vials and other working materials should not circulate among working units!

- Use always sterile pipette tips with filters
- Wear separate coats and gloves in each area
- Routinely decontaminate your pipettes and the laboratory benches with decontaminant
- Avoid aerosols

8. PROCEDURE

The complete procedure is separated in three steps:

- A) DNA extraction
- B) Amplification and combined detection of DNA fragments using TaqMan probes.
- C) Interpretation of the results using the ABI: PRISM SDS- (respectively the Stratagene: MxPRO- or Corbett Research: Rotorgene 3000/ 6000-) software.

A) DNA-EXTRACTION

- 1) DNA extraction (by use of a commercial available DNA isolation kit):
Extract bacterial DNA from clinical respiratory samples by use of a commercial DNA isolation kit (suited for throat swabs, sputum etc.) according to the manufacturer's instructions.
- 2) If (TaqMan) PCR is not performed immediately, store the extracted DNA at -20°C .

B) **Real time Chlamydia pneumoniae (TaqMan) PCR-PROTOCOL**

Please carefully read the manufacturer's instructions before starting the procedure!
Each assay should include a negative and positive control. Use filter tips for all pipetting:

- 1) Pipet **40 µl** of the amplification mix per well of a 96 wells optical microtiter plate. The number of wells used is calculated from the number of samples plus one positive and one negative control.
- 2) Add **10 µl** of sample DNA or positive or negative control per well, respectively. Mix by up and down pipetting. Pipet the negative control first. To avoid contamination, it is advisable to cover the wells containing the negative control with an adhesive seal while pipetting the positive control and sample DNA. Remove this adhesive seal after preparing all wells.
- 3) Cover the 96 wells optical microtiter plate with optical adhesive cover.
- 4) Run the PCR using following temperature protocol:

95°C for **10 min**

45 cycles of:

95°C for **15 sec**
60°C for **60 sec**

C) **PCR ANALYSIS AND INTERPRETATION OF RESULTS**

Chlamydia pneumoniae specific amplification is detected by FAM fluorescence. The internal control is measured by VIC / HEX fluorescence.

Use following settings to define a reporter and quencher with the ABI PRISM SDS software:

Detection	Reporter	Quencher
<i>Chlamydia pneumoniae</i> DNA	FAM	none
Internal Control	VIC / HEX	none

Following results can arise:

- 1) FAM fluorescence is detected.
The result is positive: The sample contains *Chlamydia pneumoniae*.
The occurrence of VIC / HEX fluorescence is inessential as high concentrations of *Chlamydia pneumoniae* DNA may reduce or even inhibit the amplification of the internal control.

- 2) No FAM, but VIC / HEX fluorescence is detected.
The result is negative: The sample does not contain *Chlamydia pneumoniae*. The detected signal of the internal control excludes the possibility of an inhibition of the PCR.

- 3) Neither FAM, nor VIC / HEX fluorescence is detected.
A diagnostic statement can not be made.
An inhibition of the PCR reaction occurred.