

Chlamydia pneumoniae TaqMan PCR Kit

Real time PCR

Cat. No.: 963100
For Research use only

INTENDED USE

The AnDiaTec® *Chlamydia pneumoniae* TaqMan PCR kit is a qualitative screening assay for the detection of *Chlamydia pneumoniae* in clinical respiratory tract samples (e.g. throat swabs, sputum, etc.) by amplification and real time detection in the microplate system of Applied Biosystems.

SUMMARY

Chlamydia pneumoniae is a widely distributed obligate intracellular pathogen causing upper and lower respiratory tract infections (e.g. 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.

PRINCIPLE OF THE TEST

The AnDiaTec® *Chlamydia pneumoniae* TaqMan PCR kit contains specific primers, TaqMan probe and additional materials for the detection of *Chlamydia pneumoniae* in clinical respiratory tract samples by polymerase chain reaction (PCR).

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 ABI's optical unit, ABI PRISM SDS. *Chlamydia pneumoniae* specific amplification is measured by FAM fluorescence.

The use of external standards allows the quantification of *Chlamydia pneumoniae* within the samples.

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*'s detection and is measured by a probe's VIC fluorescence.

REAGENTS PROVIDED

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

FOR AMPLIFICATION

Reference	Type of Reagent	Presentation	Cap Color
A1	<i>Chlamydia pneumoniae</i> Amplification Mix	3 vials á 1.5 mL	blue
A2	Positive Control	1 vial, 0.5 mL	red
A3	Negative Control	1 vial, 0.5 mL	green

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. Avoid repeated freezing and thawing of the reagents for several times. If used sporadically, prepare aliquots of the reagents.

ASSAY PROCEDURE

Required materials provided:

- * PCR Reagents
- * Product insert

Required materials not provided:

1. ABI system or a comparable instrument.
2. 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).
3. Optical adhesive covers (Applied Biosystems) or comparable covers.
4. DNA-extraction kit
5. Pipets (0.5 µL - 1000 µL)
6. Sterile filter tips for micro pipets

WARNINGS AND PRECAUTIONS

- 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).

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

- * Do not use kit after its expiration date.
- * Set up (if possible) two separate working areas:
 1. For isolation of the DNA
 2. For amplification/detection of amplification products.
- * Always use sterile pipette tips with filters.
- * Wear separate coats and gloves in each area.
- * Routinely decontaminate your pipettes and laboratory benches with decontaminant.
- * Avoid aerosols.
- * Kit contains material to perform 96 tests.

PROCEDURE

The complete procedure is separated into three steps:

1. DNA-extraction
2. Amplification and combined detection of DNA fragments using TaqMan probes (ABI system)
3. Interpretation of the results using the ABI PRISM SDS software.

1. DNA-EXTRACTION

1.1 Extraction of DNA (with commercially available DNA-Extraction Kit):

Extract genomic DNA by use of a commercial DNA isolation kit from respiratory samples (throat swabs, sputum, etc.) according to manufacturer's instructions.

1.2 If TaqMan PCR is not performed immediately after extraction, store extracted DNA at -20°C.

2. Real time TaqMan Chlamydia pneumoniae PCR Protocol

Please carefully read the manufacturer's instructions before starting the PCR!

Each assay should include a negative and positive control.

Use filter tips for all pipetting:

2.1 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.

2.2 Add 10 µL of sample DNA or positive and 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.

2.3 Cover the 96 wells optical microtiter plate with optical adhesive cover.

2.4 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.

3. PCR-ANALYSIS AND INTERPRETATION OF RESULTS

Chlamydia pneumoniae specific amplification is detected by FAM fluorescence. The internal control is measured by VIC 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	none

The following results may occur:

3.1 FAM fluorescence is detected.

The result is positive. The sample contains *Chlamydia pneumoniae*.

The occurrence of VIC fluorescence is inessential as high concentration of *Chlamydia pneumoniae* DNA may reduce or even inhibit the amplification of the internal control.

3.2 No FAM, but VIC 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.

3.3 Neither FAM, nor VIC fluorescence is detected.

A diagnostic statement cannot be made.

An inhibition of the PCR reaction occurred.

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