CE 0483

ASTRA BIOTECH

PCR - Chlamydia trachomatis

Real Time PCR test for detection of *Chlamydia trachomatis* DNA

Instructions for use

IVD

Kit PCR per Chlamydia trachomatis	Σ	*112 test* *112 tests*	**Form S 84-09** **Form T 84-10** REF
Kit PCR per Chlamydia trachomatis	Σ	*56 test* *56 tests*	**Form S 84-09-56** **Form T 84-10-56** REF
Kit PCR per Chlamydia trachomatis	Σ	*24 test* *24 tests*	**Form S 84-09-24** **Form T 84-10-24** REF

LEGENDA DEI SIMBOLI / *SYMBOLS LEGEND*

IVD — Dispositivo medico diagnostico in vitro
In Vitro Diagnostic Medical Device

REF — Numero di catalogo
Catalogue number

 Scadenza
Use by

 Data di produzione
Date of manufacture

 Proteggere dalla luce
Protect from light

 Quantità sufficiente per < 112/56/24 > test
Contains sufficient for < 112/56/24 > tests

 Consultare la metodica
Consult operating instructions

NC — Controllo Negativo
Negative Control

Taq — Taq DNA-polimerasi
Taq DNA-polymerase

PC — Controllo Positivo
Positive Control

 Dichiarazione di conformità CE
EC Declaration of conformity

LOT — Lotto
Batch code

 Produttore
Manufacturer

 Limitazione della temperatura
Temperature limitation

 Mantenere asciutto
Keep dry

 Attenzione, consultare i documenti di accompagnamento
Caution, consult accompanying documents

Ct-Mix-T — PCR-Mix *C. trachomatis*, Form T

Ct-Mix-S — PCR-Mix *C. trachomatis*, Form S

IC — Controllo Interno
Internal Control

3. MATERIALS PROVIDED

3.1 Form S (liquid reaction mix aliquoted into 0.2 mL low profile 8-tube strips; DNA polymerase in a separate tube).

Label	Component	84-09		84-09-56		84-09-24	
		Volume, µL	Quantity	Volume, µL	Quantity	Volume, µL	Quantity
Ct-Mix-S	PCR-Mix *C. trachomatis*, Form S	10	14×8× 0.2 mL tubes	10	7×8×0,2 mL tubes	10	3×8×0,2 mL tubes
Taq	Taq DNA-polymerase	1120	1 tube	560	1 tube	240	1 tube
PC	Positive Control	250	1 tube	250	1 tube	250	1 tube
IC	Internal Control	1120	2 tubes	1120	1 tube	1120	1 tube
NC	Negative Control	1500	1 tube	1500	1 tube	1500	1 tube

The Form S of the product was validated to be usedwith the following real-time PCR cyclers: CFX96, / Light Cycler 96 (LC 96) and DTprime (corresponds to DTlite, SaCycler, AstraCycler 48 and AstraCycler 96) While using other real-time PCR cyclers compatible with low profile PCR tubes, contact the manufacturer for more information concerning compatibility.

3.2 Form T (liquid reaction mix aliquoted into 0.2 mL regular profile reaction tubes; DNA polymerase in a separate tube).

Label	Component	84-10		84-10-56		84-10-24	
		Volume, µL	Quantity	Volume, µL	Quantity	Volume, µL	Quantity
Ct-Mix-T	PCR-Mix *C. trachomatis*, Form T	10	112×0.2 mL tubes	10	56×0.2 mL tubes	10	24×0.2 mL tubes
Taq	Taq DNA-polymerase	1120	1 tube	560	1 tube	240	1 tube
PC	Positive Control	250	1 tube	250	1 tube	250	1 tube
IC	Internal Control	1120	2 tubes	1120	1 tube	1120	1 tube
NC	Negative Control	1500	1 tube	1500	1 tube	1500	1 tube

The Form T of the product was validated to be usedwith the following real-time PCR cyclers: Rotor-Gene Q (corresponds to Rotor-Gene 3000/6000). While using other real-time PCR cyclers compatible with regular profile PCR tubes, contact the manufacturer for more information concerning compatibility.

4. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Biosafety cabinet;
- Real-Time PCR cycler: CFX-96 , DTprime (corresponds to DTlite, SaCycler, AstraCycler 48 and AstraCycler 96), Light Cycler 96, Rotor-Gene Q (corresponds to Rotor-Gene 3000/6000);
- Vortex mixer;
- Microcentrifuge (suitable for 0.2 mL tubes, max. 2400 rpm);
- two separate pipette sets for DNA-free and DNA-containing components;
- Filter tips (10, 100; 200 and 1000 µL);
- Disposable safe-seal 1.5 mL microcentrifuge tubes
- Separate working areas for DNA extraction (Sample preparation area), PCR preparation (Reagent preparation area) and for Real-Time PCR amplification (Amplification area). Each area must be fitted with separate set of pipettes, instruments and laboratory clothing.
- Multi DNA extraction kit (Ref 80-01) or Rapid DNA Extraction kit (Ref 80-02).

5. STORAGE CONDITIONS AND STABILITY OF THE KIT

The kits are designed to perform 112 (Ref. 84-09/10), 56 (Ref. 84-09-56/10-56) or 24 (Ref. 84-09-24/10-24) reactions, including controls. ***Chlamydia trachomatis*** **PCR kit** should be stored at -18…-30 °C, preferably in the original kit box until the expiration date. The manufacturer guarantees the functionality of the kit components up to a number of 15 freezing and thawing cycles.

. The expiry date of the kit is stated on the box label, expiry date for each component is indicated on the respective label. Storage at +25 °C is allowed for no more than 7 days (any kit format).

6. SPECIMEN COLLECTION, STORAGE AND TRANSPORT

Total DNA can be extracted from urethral swab specimens, endocervical and vaginal smears, urine samples.

Type of specimen	Collection*	Storage-Transport**
urethral swab, endocervical and vaginal smears	Spicemens collected and transported in 0,5 mL of 0.9 % NaCl solution or other transport medium entitled for this purposes	• At +18…+25 °C for ≤ 48 hours • Refrigerated at 2-8°C for ≤ 4 days • Frozen at -18…-35 °C > 4 days
Urine	first 10 mL to 50 mL of urine, obtained ideally between 2 h and 6 h after the last micturition in a sterile screw-cap container without preservative	• At +18…+25 °C for ≤ 48 hours • Refrigerated at 2-8°C for ≤ 2 weeks • Frozen at -18…-35 °C > 2 weeks

*Standard collection procedures are appropriate to obtain specimens.

**Ensure that the transport of human specimens meets all local and national regulations for the transport of etiologic agents.

7. ASSAY PROCEDURE

Positive and Negative Controls must be included in each run.

The complete procedure consists of three stages:

I. DNA Extraction
II. Real-Time PCR
III. Data analysis and interpretation

I. DNA extraction

NOTE: *The quality of the extracted DNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for DNA extraction is compatible with real-time PCR technology. Chlamydia trachomatis PCR kit was validated with the following kits and systems for DNA extraction, which are strongly recommended by the manufacturer:*

- *Multi DNA extraction kit (Astra Biotech, Ref 80-01)*
- *Rapid DNA Extraction kit (Astra Biotech, Ref 80-02)*

The compatibility of other DNA extraction procedure for use with Chlamydia trachomatis PCR kit has to be validated by the user.

1) Take **IC** out from the kit to thaw at room temperature, the rest components place back in the freezer. Gently vortex **IC** and centrifuge for 2-3 s.

2) Prepare and mark required quantity of 1.5 mL microcentrifuge tubes equal to the number of samples + 1 tube for negative extraction control and label as “NEC”.

NOTE: *In case of using Rapid DNA Extraction kit, prepare and mark required quantity of 1.5 mL microcentrifuge tubes with lysis buffer from the extraction kit.*

NOTE: *Each extraction procedure should be performed using “NEC”.*

3) If Astra Biotech DNA extraction kit is used, ignore steps 4) - 6) and follow the Astra Biotech DNA extraction kit manual; otherwise continue with step 4).

4) Place **20 µL IC into each** 1.5 mL microcentrifuge **tube**.

5) Add **100 µL NC into** the microcentrifuge **tube marked "NEC**".

NOTE: *The Elution buffer contained in DNA extraction kit can be used instead of* **NC**.

6) Follow the manual of the used DNA extraction kit.

II. Real-Time PCR

Kit components preparation:

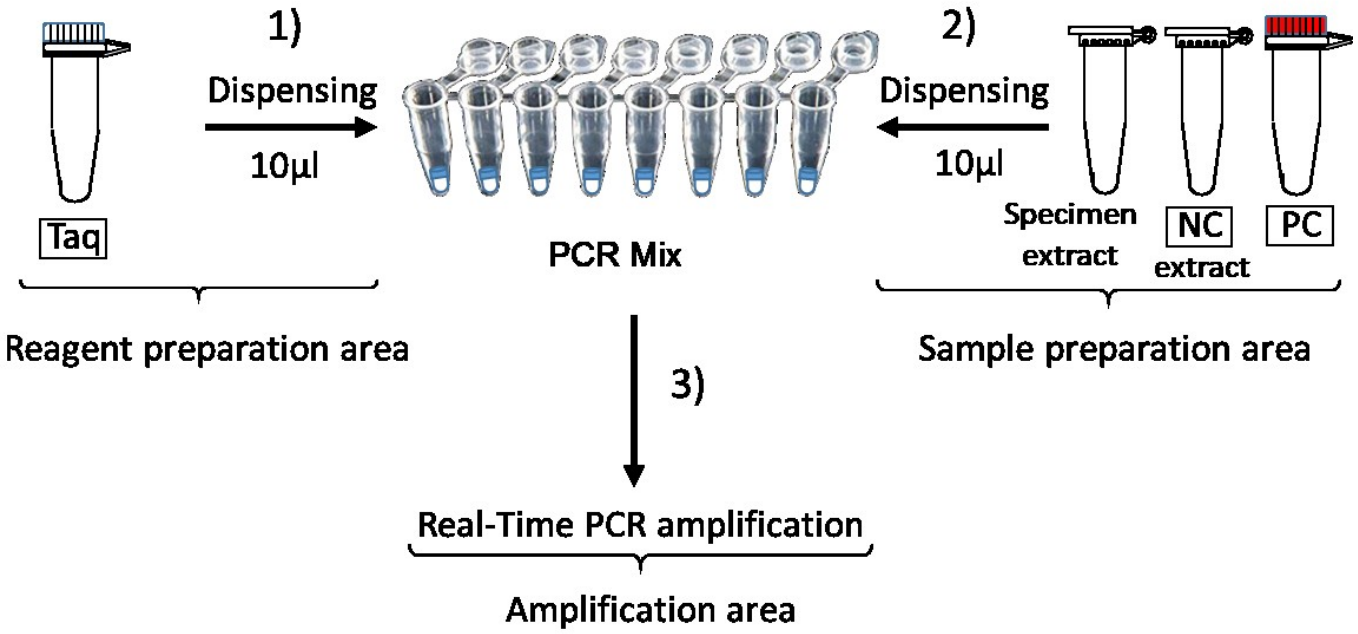
1) Take out from the freezer and thaw the following components of the kit:

- **PC** (positive control)
- **Taq** (Taq polymerase)
- required quantity of 0.2 mL microcentrifuge tubes/wells with **Ct-Mix-S/T** equal to the number of samples + 1 tube/ well for "PC" + 1 tube/well for "NEC".

The rest components place back in the freezer.

2) Hand over **PC** to the Sample preparation area intended for DNA extraction and its dispensing.

Workflow



1) PCR mix preparation

***NOTE**: PCR mix preparation has to be performed in the Reagent preparation area. Do not prepare the PCR mix in the same area as the DNA extraction or amplification to prevent DNA contamination.*

1. Mark prepared PCR tubes with **Ct-Mix-S/T**.
   ***NOTE:** Different positions for marking the tubes are necessary because of differences in the fluorescence detection: When using CFX96/96 Touch, LC96 or SaCycler (DTprime) Real-Time PCR cyclers mark tubes outside the center of tube cap or on the tube side. When using Rotor-Gene Q instruments mark the tubes on the caps.*
2. Gently vortex **Taq** and centrifuge at 1500-2400 rpm for 2-3 s.
3. Place 10 µl of **Taq** into each tube avoiding tip contact with paraffin.

2) Dispensing of test samples and the controls

***NOTE**: Dispensing of test samples and the controls have to be performed in the Sample preparation area.*

***NOTE**: Use a separate filter tip for each DNA sample.*

1. Centrifuge the DNA extracted from the specimens and “NEC” (**NC** extract) at 13000 rpm for 1 min just before adding them to the PCR tubes.
2. Add 10 µl of DNA extracted from specimens and “NEC” to separate PCR tubes avoiding tip contact with paraffin.
3. Vortex and centrifuge the **PC** at 1500-2400 rpm for 2-3 s.
4. Add 10 µL of **PC** to the PCR tube marked “PC”.
5. Close the tubes and centrifuge at 1500-2400 rpm for 2-3 s.

3) Real-Time PCR Amplification:

NOTE*: Real-Time PCR Amplification has to be performed in the Amplification area. Separate room for this area is recommended.*

- Load tubes into a Real-Time PCR cycler.
- Fill in the location of the samples. Set up fluorescent detection on the channels ROX/ Orange and FAM/ Green.
- Run the PCR protocol.

Stage	Temperature, °C	Data collection	Time	Number of cycles
Hold	94	-	3 min	1
Cycling	94	-	10 s	5
	60		20 s	
Cycling	94	-	10 s	45
	60	ROX/ Orange FAM/ Green	20 s	

- Follow the manufacturer instruction before using the PCR Real-Time cycler.

III. Data Analysis

1) <u>General conditions</u>:

ROX dye is used to detect the *Chlamydia trachomatis* DNA and FAM is used to detect the **IC** DNA.

Analysis of the results can be performed by means of a Real-Time PCR thermal cycler's software in accordance to its instruction. For the validated cyclers detailed instructions concerning protocol set up and results analysis are given in «Astra Biotech: Real-Time PCR kits Guidelines».

Analysis of the results can be performed only by using following parameters: Cq **Ct**, Cq **PC** and Cq **IC**, specified for each lot in the Quality control Sheet provided with every kit.

Cq **Ct** = critical value of quantification cycle for *C. trachomatis* in the ROX channel,is crucial for detection positive results;

Cq **PC** = *critical value of quantification cycle for Positive Control* (PC) in the ROX channel is used to determine whether kit components work properly (general control of the reagents effectiveness),is crucial for detection negative results

Cq **IC** = *critical value of quantification cycle for Internal Control* (IC) in the FAM channel is used to control all stages of the analysis (overall effectiveness) in each tube, including inhibition and DNA loss.

The results of single run are valid if:

- Cq PC ≤ Cq **PC** in the ROX channel. Nevertheless, if Cq value for PC is determined as > Cq **PC**, in that case all the specimens defined as positive in this run should be considered as valid, whereas all the specimens defined as negative should be considered as invalid.
- Cq of NEC > Cq **Ct** in the ROX channel and Cq of NEC ≤ Cq **IC** in the FAM channel, but there are some exclusions, for more information see 2).

2) Data interpretation

Specimen is considered as positive on DNA of *C. trachomatis*:

Cq ≤ Cq **Ct** in the ROX channel,

even if Cq > Cq **IC** in the FAM channel or is not determined.

Specimen is considered as negative on DNA of *C. trachomatis*:

Cq > Cq **Ct** in the ROX channel or is not determined

Cq ≤ Cq **IC** in the FAM channel,

Cq of PC ≤ Cq **PC** in the ROX channel.

Specimen is considered as invalid:

Cq> Cq **Ct** in the ROX channel and at least one of this conditions are satisfied:

- Cq > Cq **IC** in the FAM channel or is not determined.
- Cq of PC > Cq **PC** in the ROX channel or is not determined.

In case of contamination:

Cq of NEC ≤ Cq **Ct** in the ROX channel. Measures must be taken to eradicate the source of contamination. In case of contamination, all positive specimens satisfying condition Cq ≥ (Cq of NEC – 5) in the ROX channel are considered as invalid, the other results are considered as valid. For all specimens with invalid results it is recommended to perform the analysis from the DNA extraction stage.

Data interpretation, summary table:

Cq ROX Detection of *C.t. DNA* **for sample**	Cq FAM Detection of IC DNA **for sample**	Cq **ROX** Detection of *C.t. DNA* **for PC**	Cq **ROX** Detection of *C.t. DNA* **for NEC**	Result
Cq≤ Cq **Ct**	any	any	Cq > Cq **Ct** or N/A	Positive
Cq > Cq **Ct** or N/A	Cq≤Cq*IC*	Cq ≤ Cq **PC**	any	Negative
Cq > Cq **Ct** or N/A	Cq > Cq **IC** or N/A	any	any	Invalid
Cq > Cq **Ct** or N/A	any	Cq > Cq **PC** or N/A	any	
Cq ≤ Cq **Ct** *and* Cq > Cq NEC – 5	any	any	Cq ≤ Cq **Ct**	
Cq ≤ Cq **Ct** *and* Cq ≤ Cq NEC – 5	any	any	Cq ≤ Cq **Ct**	Positive

Abbreviations:

Cq **Ct** *critical value of quantification cycle for Chlamydia trachomatis*

Cq **IC** *critical value of quantification cycle for Internal Control (IC)*

Cq **PC** *critical value of quantification cycle for Positive Control (PC)*

Cq **Ct**, Cq **IC**, Cq **PC** are specified for each lot in the Quality control Sheet provided with every kit

NEC negative extraction control

Cq of NEC *quantification cycle for NEC in the ROX channel is determined in case of contamination*

N/A *Cq value is not determined*

8. PERFORMANCE CHARACTERISTICS OF THE KIT

Analytical sensitivity:

The detection limit of the kit evaluated via probit analysis is 600 copies of *C. trachomatis* genomic DNA per 1 mL (i.e. 10 copies of genomic DNA per reaction). This limit is defined for confidence level 95%. It is assumed that 100 µl of specimen was sampled for DNA extraction and elution volume was 60 µl.

Analytical specificity:

A false-positive reaction is absent with standard human DNA samples and standard DNA samples of *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis, Mycoplasma genitalium, Gardnerella vaginalis*, *Lactobacillus spp*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Trichomonas vaginalis*, *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, *Neisseria gonorrhoeae*, *Neisseria flava*, *Neisseria subflava*, *Neisseria mucosa*, *Neisseria sicca*, *Treponema pallidum*, *Toxoplasma gondii*, *Candida albicans*, *HSV* type 1 and 2, *CMV and HPV*.

9. LIMITATIONS OF THE METHOD

Any clinical diagnosis should not be based on the results of *in vitro* diagnostic methods alone. For diagnosis establishment a physician is supposed to consider all the available clinical and laboratory findings.

10. SAFETY PRECAUTIONS

- **This kit is for in vitro diagnostic use only.** The operator should thoroughly follow the manual to obtain reliable data. This instruction manual is valid only for the present kit with the listed contents. Any exchange of the kit components is not allowed by CE regulations.
- Test should only be performed by skilled personnel considering GLP (Good Laboratory Practice) guidelines.
- Don't use the kit after its expiration date.

- Do not pool reagents from different lots or from different tubes of the same lot (the only exception for **IC** is described below). Immediately after use, close all tubes in order to avoid leakage.
- In case of simultaneous using of two or more Astra Biotech Infectious Real-Time PCR kits for detection of different infectious agents, **IC** with the latest lot number can be used for all of these kits.
- We recommend setting up blank protocols of PCR before the analysis.
- PCR technology is extremely sensitive. The amplification of a single DNA molecule generates millions of identical copies. Therefore, set up three separate working areas for a) sample preparation b) PCR reagent preparation and c) PCR amplification. For each working area a different set of pipettes and protective clothing should be reserved.
- Use sterile filter tips for pipetting and use special PCR pipettes for aerosol free pipetting.
- Do not use the same tip for two different components, neither DNA-free nor DNA containing.
- Routinely decontaminate your pipettes and the laboratory benches.
- After usage, all the reagents and test components should be discarded as consisted with local statements.
-  Source materials of animal origin used for kit components preparation were found safe for humans. However, none of known laboratory test guarantees total absence of the hazard agents. Therefore, all kit components and patient's samples should be handled as potentially hazardous.
-  After usage the kit components, specimens and all consumables which contacted with specimens during handling, storage or assay (tubes, vials, gloves, pipette tips etc.) should be collected separately and sterilized by autoclaving or disinfectant treatment. After sterilization all components and expendable materials may be utilized as non-dangerous garbage. Other

components of the kit should be discarded into conventional garbage.

-  The following precautions should be taken:
  - do not smoke, eat or drink while performing the assay;
  - always use protective gloves while performing the assay;
  - never pipette material by mouth;
  - be careful while handling **PC**, avoid its splashing;
  - in case of spilling, wipe up the spills promptly and wash affected area thoroughly using decontaminant.
- GLP including all general and individual regulations should be applied for the kit usage.

11. REFERENCE

Jaton K1., Bille J., Greub G., “A novel real-time PCR to detect Chlamydia trachomatis in first-void urine or genital swabs” // J Med Microbiol. 2006 Dec;55(Pt 12):1667-74.

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