

A decorative graphic consisting of numerous thin, parallel blue lines that curve and overlap to form a large, smooth, wave-like shape in the upper half of the page.

DiAGSure Chlamydia Infection Detection Kit

20 Tests

For research use only

Description:

Chlamydia infection which is also known as Chlamydia is a sexually transmitted disease caused by the bacterium *Chlamydia trachomatis*. It can affect both men and women. Infants get also affected. The disease can be spread while having sex. It can be passed during child birth from infected mother to the baby.

Symptoms in women are vaginal discharge or burning with urination. Symptoms in men may include burning with urination, discharge from the penis, pain and swelling of one or both testicles. Sometimes the infection can spread to the upper genital tract of the women which can cause future infertility or ectopic pregnancy. It can also infect eyes, without treatment which can cause trachoma which is a common cause of blindness. Chlamydia can sometimes develop reactive arthritis, which can affect both sexes but more common in male.

One of the best ways to diagnose this disease is nucleic acid based Polymerase Chain Reaction (PCR). Those who are infected are recommended to undergo the tests for HIV, gonorrhoea and syphilis.

Disclaimer: DiAGSure Chlamydia infection Detection Kit is an in-vitro diagnostic PCR Based detection of *Chlamydia trachomatis* pathogen in human clinical samples.

Intended Use:

This kit detects a conserved 144-bp region in the genome of *Chlamydia trachomatis* pathogen. This kit also contains an internal

control which is set-up in a separate tube and amplifies a 221-bp region from of human DNA. This internal control has been included to ensure proper DNA extraction and PCR reaction in the absence of amplification in the target sequence.

Principle:

The DiAGSure Chlamydia infection Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *Chlamydia trachomatis* specific 144 bp region in the pathogenic genome using gene-specific primers. PCR-based detection is emerging as a highly sensitive diagnostic tool for the detection of pathogen in a wide array of clinical samples. A basic PCR reaction involves three basic steps:

- i. Denaturation, where separation of the two DNA strands occur
- ii. Annealing, where the primers are allowed to anneal to their cognate templates
- iii. Extension, where the actual amplification occurs that is repeated between 25 and 40 cycles in each assay. The PCR primers have been designed to ensure high specificity and sensitivity.

Features:

- ✓ Fast and simple
- ✓ Rapid detection of the pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the *C. trachomatis*

- ✓ Reproducibility of results

Storage and Shelf life:

The provided kit has a shelf-life of 6 months when stored at -20°C. Repeated thawing and freezing of PCR reagents may reduce the sensitivity and therefore should be avoided. If reagents are to be used multiple times, we recommend storing reagents as aliquots to avoid repeated freeze and thaw. The degradation of sample DNA specimens may also compromise with the sensitivity of the assay. Usage of the kit after the expiry date stated on pack is not recommended.

Kit contents:

(Storage: -20°C, in a Frost-free freezer):

Kit Contents	Vial's cap colour	Volume for 20 tests
DiAGPol PCR Master Mix	Red	1.4mL
<i>C.trachomatis</i> primer mix	Green	45 µL
Internal control primer mix	Green	25 µL
DiAGSure DNA ladder	Yellow	100 µL
Gel loading dye	White	100 µL
Nuclease free water	White	500 µL

Sample Material Preparation:

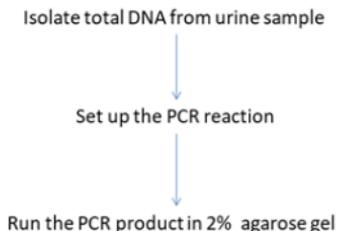
The DiAGSure Chlamydia infection Detection Kit detects the presence of *C.trachomatis* in human urine samples. Isolate total DNA from urine (which includes pathogenic DNA in case of infected samples). Use a specified amount (see below) of this DNA to amplify the pathogenic gene.

Amount of sample (urine) - 5 mL

Final elution volume of extracted DNA - 50 µL

Note: The entire process has to be performed within 1-2 days of sample collection. Sample has to be stored at -80°C.

Basic workflow:



DNA Isolation:

The initial amount of urine sample 5uL. Isolate the DNA and Check the quality by running it in a 1% agarose gel and quantitating it in a

nano-drop instrument (Thermo-Scientific) or a uv-visible spectrophotometer.

PCR Protocol:

Preparation of template DNA: Take 9 μL of autoclaved water, to that add 1 μL of isolated genomic DNA from urine. Vortex gently and spin down the contents at the bottom of the tube.

Plan your work mark and add the following reagents in the indicated order to a 0.2 ml PCR tube and mix vigorously by pipetting up and down. Pulse spin to bring the contents at the bottom of the tube.

Set up a 20 μL PCR reaction by adding the following constituents in a PCR tube:

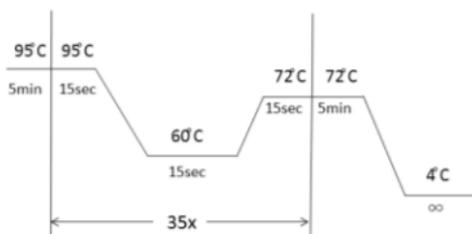
Components	Amount in Sample tube (+)	Amount in NTC tube (-)
DiAGPol PCR Master Mix	18 μL	18 μL
<i>C.trachomatis</i> Primer Mix	1 μL	1 μL
Template DNA	1 μL	-
Nuclease Free Water	-	1 μL

(If required amount of template DNA can be changed accordingly. Preferably use barrier tips.)

Mix vigorously by pipetting up and down and pulse-spin to bring the contents at the bottom of the tube and place the tube in following thermal cycling program.

PCR conditions:

Stage	Temperature (°C)	Time	No. of cycles
Initial denaturation	95	5 mins	1
Denaturation	95	15secs	35
Annealing	60	15secs	
Extension	72	15secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



PCR cycling conditions

Add 1µL of the supplied gel-loading dye to the PCR products, mix well and run the PCR products along with 5µL of the supplied DiAGSure DNA ladder in a 2% agarose-TAE gel.

Note: To ensure proper DNA isolation, PCR amplification of isolated genomic DNA can be done with the internal control primer mix, provided along with the kit.

Results Interpretation:

The presence of a 144-bp product in the sample lane Indicates the presence of *C.trachomatis* DNA in the sample.(Fig1)

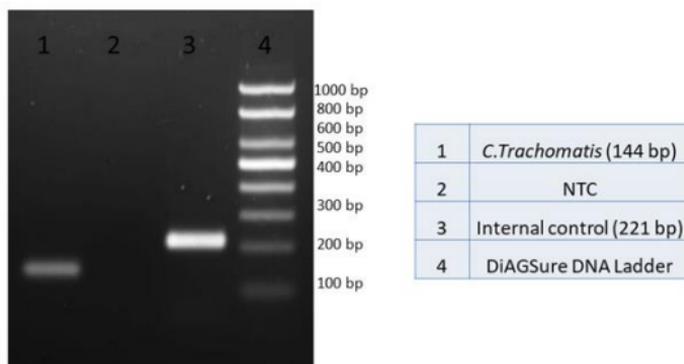


Fig.1

Representative gel image of 144 bp (*C. trachomatis*) along with DiAGSure DNA ladder.(band for internal control has also been shown).

Sensitivity:

The kit can identify 1.8 attomoles of *Chlamydia trachomatis* genomic DNA.

Quality Control:

All reagents in the DiAGSure Chlamydia Infection Detection Kit are free from endonuclease and exonuclease activities and the kit has been functionally tested for amplification

Safety information:

The DiAGSure Chlamydia Infection Detection Kit is for laboratory use only. Use proper safety measures while handling clinical samples, like wearing mask, gloves, lab-coat, etc.

Technical assistance:

Satisfaction of the customers is our utmost priority. For any kind of technical assistance, always feel free to reach out to us at tech.support@gccbiotech.co.in.