

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 Toxoplasmosis Detection Kit**

---

**20 Tests**

*For research use only*

## Description:

---

**Toxoplasmosis** is a parasitic disease caused by *Toxoplasma gondii*. In adults, infections with toxoplasmosis usually cause no obvious symptoms. Sometimes, people may have a few weeks or months of mild, flu like illness, such as muscle aches and tender lymph nodes. Eye problems may develop in a small number of people..

This disease is usually spread by eating poorly cooked food, exposure to infected cat feces, and from a mother to a child during pregnancy if the mother becomes infected. Rarely, the disease is spread by blood transfusion. It is not otherwise spread between people. The parasite is only known to reproduce sexually in the cat family. However, it can infect most types of warm-blooded animals, including humans. Up to half of the world's population is infected by toxoplasmosis, but have no symptoms.

Disclaimer: DiAGSure Toxoplasmosis Detection Kit is an in-vitro diagnostic PCR Based detection of *Toxoplasma gondii* in human clinical samples.

## Intended Use:

---

This kit detects a conserved 132-bp region in the genome of *T.gondii* 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 Toxoplasmosis Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *T.gondii* specific 132 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 *T. gondii*
- ✓ 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>T.gondii</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

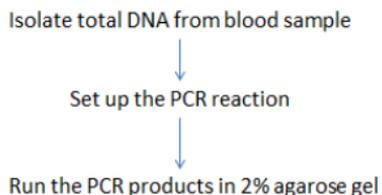
### Sample Material Preparation:

The DiAGSure Toxoplasmosis Detection Kit detects the presence of *Toxoplasma gondii* in human blood samples. Isolate total DNA from

blood. Use a specified amount (see below) of this DNA to amplify the *Toxoplasma gondii* gene.

## Basic workflow:

---



Starting volume of blood: 200 $\mu$ L

Elution volume: 30 $\mu$ L

## PCR Protocol:

---

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 to the bottom of the tube.

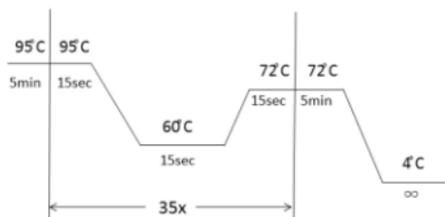
Set up a 20 $\mu$ L PCR reaction by adding the following constituents in a PCR tube:

Components	Amount in Sample tube(+)	Amount in NTC tube(-)
DiAGPol PCR Master Mix	18 $\mu$ L	18 $\mu$ L
<i>T. gondii</i> Primer Mix	1 $\mu$ L	1 $\mu$ L
Template DNA	1 $\mu$ L	0 $\mu$ L
Nuclease Free Water	0 $\mu$ L	1 $\mu$ L

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

### PCR conditions:

Stage	Temperature ( $^{\circ}$ 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	$\infty$	1



Diagrammatic view of 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.

### Results Interpretation:

The presence of a 132-bp product in the sample lane indicates presence of *Toxoplasma gondii* DNA in the sample (Fig 1).

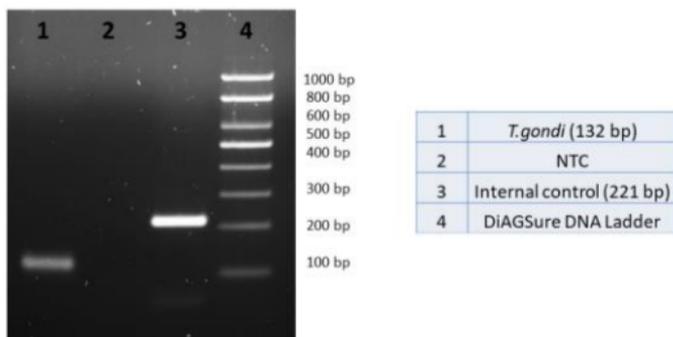


Fig 1. Representative gel image showing amplification of the *T.gondii* pathogenic genes.

### Sensitivity:

The kit can identify 1.8 attomoles of the respective genomic DNA.

### Quality Control:

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

### **Safety information:**

---

The DiAGSure Toxoplasmosis 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](mailto:tech.support@gccbiotech.co.in).