

# **DiAGSure Amoebiasis Detection Kit**

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**20 Tests**

*For research use only*

## **Description:**

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Amoebiasis is a very common infection which occurs in the gastro-intestinal tract of human. Any of the amoeba of the *Entamoeba* group is responsible for the infection, out of which *Entamoeba histolytica* is the most common. Sometimes it can be present with no, mild or severe symptoms. About 90% of the infected people are asymptomatic but it can become more serious. Symptoms are abdominal pain, diarrhoea and bloody diarrhoea. Sometimes the situation can be more complicated. Due to loss of blood the people may suffer from anemia.

The disease occurs when the amoeba comes in contact with the cells lining the intestine. Then it secretes some substances including enzymes to destroy bacterial proteins and cell membranes which lead to ulceration of the intestine.

Microscopy is the most widespread method to diagnose this disease across the world by stool examination, but it is not so sensitive and accurate. Polymerase Chain Reaction is considered as the best way for diagnosis of this disease.

Disclaimer: DiAGSure Amoebiasis Detection Kit is an in-vitro diagnostic PCR Based detection of *Entamoeba histolytica* pathogen in human clinical samples.

## Intended Use:

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This kit detects a conserved 308-bp region in the genome of *Entamoeba histolytica* pathogen. This kit also contains an internal control which is set-up in a separate tube and amplifies a 520-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:

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The DiAGSure Amoebiasis Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *Entamoeba histolytica* specific 308 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:

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- ✓ Fast and simple

- ✓ Rapid detection of the pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the *E. histolytica*
- ✓ 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>E. histolytica</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:

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The DiAGSure Amoebiasis Detection Kit detects the presence of *E. histolytica* in human stool samples. Isolate total DNA from stool. Use a specified amount (see below) of this DNA to amplify the pathogenic gene.

Amount of sample (Stool) – 50 mg

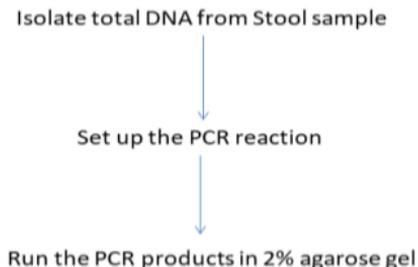
Final elution volume of extracted DNA - 50 µL

(Before DNA isolation the sample must undergo the required steps to reduce the amount of PCR inhibitors).

**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:

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## PCR Protocol:

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**Preparation of template DNA:** Take 9  $\mu\text{L}$  of autoclaved water, to that add 1  $\mu\text{L}$  of isolated genomic DNA from stool. 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 $\mu\text{L}$  PCR reaction by adding the following constituents in a PCR tube:

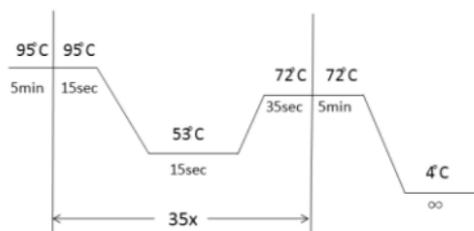
Components	Amount in Sample tube (+)	Amount in NTC tube (-)
DiAGPol PCR Master Mix	18 $\mu\text{L}$	18 $\mu\text{L}$
<i>E. histolytica</i> Primer Mix	1 $\mu\text{L}$	1 $\mu\text{L}$
Template DNA	1 $\mu\text{L}$	0 $\mu\text{L}$
Nuclease Free Water	0 $\mu\text{L}$	1 $\mu\text{L}$

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 ( $^{\circ}\text{C}$ )	Time	No. of cycles
Initial denaturation	95	5 mins	1

Denaturation	95	15secs	
Annealing	53	15secs	35
Extension	72	35secs	
Final extension	72	5 mins	1
Final hold	4	∞	1



Diagrammatic view of PCR cycling conditions

Add 1 $\mu$ L of the supplied gel-loading dye to the PCR products, mix well and run the PCR products along with 5 $\mu$ L 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:

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The presence of a 308-bp product in the sample lane indicates presence of *Entamoeba histolytica* DNA in the sample (Fig 1).

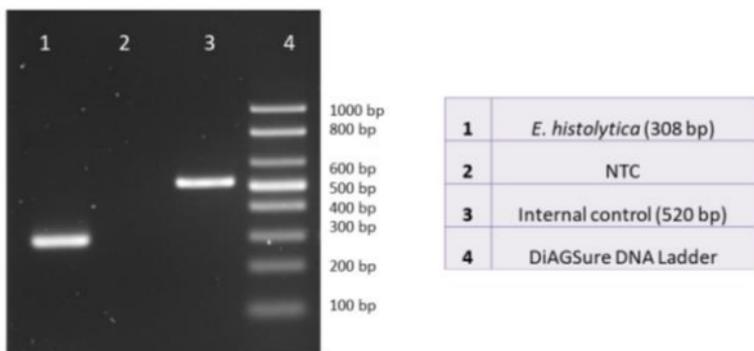


Fig.1: Representative gel image of 308 bp (*E. histolytica*) along with DiAGSure DNA ladder.(band for internal control has also been shown).

## Sensitivity:

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The kit can identify 0.2 attomoles of *Entamoeba histolytica* genomic DNA.

## Quality Control:

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

### **Safety information:**

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

### **Technical assistance:**

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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).