DiAGSure Malaria Detection Kit
20 Test

Description:

Malaria is a mosquito-borne infectious disease caused by *Plasmodium* parasite. It is mainly caused by infection with four *Plasmodium* species*: Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae, Plasmodium ovale*. Out of which *P. falciparum and P. vivax* are the most common and *P. falciparum* is most deadly.
Although *P. falciparum* traditionally accounts for the majority of deaths, recent evidence suggests that *P. vivax* malaria is associated with potentially life-threatening conditions about as often as with a diagnosis of *P.falciparum* infection. If not treated within 24 hours, *P. falciparum* malaria can progress to severe illness often leading to death. Early diagnosis and treatment of malaria reduces disease and prevents deaths. It also contributes to reducing malaria transmission.
DiAGSure Malaria Detection Kit is an in-vitro diagnostic PCR Based detection of *Plasmodium* vivax and *Plasmodium falciparum* in human clinical samples.

Principle:

The DiAGSure Malaria Detection Kit is based on semi-quantitative end-point PCR based detection of a conserved *Plasmodium* specific 235 bp region in the *Plasmodium* genome using gene-specific primers. It can also specify whether the disease is caused by *P.falciparum* or *P .vivax*, which will be really helpful for further

treatment procedure. 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 Malaria pathogen in clinical samples
- ✓ Highly sensitive
- ✓ Specific detection of the *P.falciparum , P.vivax*
- ✓ 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:

Table for kit of 20 tests:

Part 1 of 3 (Storage: Room temperature)

GSure Blood DNA Mini kit (Cat. No. G4625) – 20preps

Part 2 of 3 (Storage: Room temperature)

Kit Contents	Catalog No.	Volume for 20 tests
Agarose	G4652	20 g
GPure TAE Buffer, 40X	GS1006	50 mL
0.2mL PCR tubes		750 no.s
1.5mL microcentrifuge tubes		100 no.s
Ethidium Bromide Solution (10mg/ml)	GCR-28	500µL

N.B.: On receipt, store the Ethidium bromide solution at 4°C.

Part 3 of 3 (Storage: -20°C)

Kit Contents	Catalog No.	Vial's cap colour	Volume for 20 tests
PCR reagent mix(2x)	-	Red	1.5 ml X 2
Universal primer	G7117	Green	50 µL

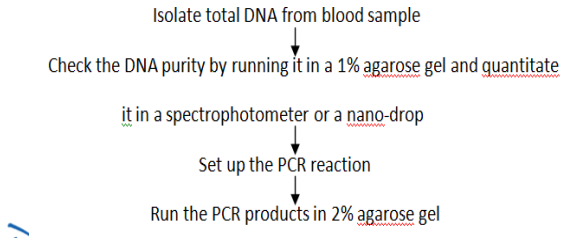
mix			
***P.vivax* primer mix**		Green	50 µL
***P.falciparum* primer mix**		Green	50 µl
Internal control primer mix	GCR-19	Green	30 ul
DiAGSure DNA ladder		Yellow	100 uL
6x gel loading dye		White	500 ul
Nuclease free water	GCR-30	White	1.5mL X 2

Protocol:

Sample Material Preparation:

The DiAGSure Malaria Detection Kit detects the presence of Malaria pathogen in human blood samples. Isolate total DNA from blood (which includes bacterial DNA in case of infected samples) using GSure Blood DNA isolation kit provided with this kit. Use a specified amount (see below) of this DNA to amplify the *Plasmodium* gene.

Basic workflow:



DNA Isolation Protocol:
Total DNA Extraction from blood:

1. To 200µL of fresh blood, 1mL of RBC lysis buffer is added, mixed thoroughly by vortexing and incubate on ice for 15mins with intermittent vortexing.
2. Centrifuge at 8000rpm for 10mins. The supernatant is discarded and the whitish pellet is again dissolved in 400µL of RBC lysis buffer followed by incubation on ice for another 15mins with intermittent vortexing.
3. Centrifuge at 8000rpm for 10mins again.
4. Discard the supernatant and resuspend the pellet in 250µL of GDBL1 by vigorous vortexing. Incubate the tube at 70°C for 15mins with vortexing every 2-3mins.
5. Add 250µL of GDBL2 buffer and mix well by inverting the tubes 6-8 times. Place the tubes again at 70°C for 15mins.
6. Add 350µL of GDBL3 buffer and invert the tubes immediately. Mix well. (Do not vortex.)
7. Centrifuge at 13000rpm for 10mins.
8. Apply the supernatant to the GMini Spin column with the aid of a pipette.
9. Centrifuge at 13000rpm for 1min and discard the flow-through.
10. Wash the column twice with 600µL of wash buffer (reconstituted) by centrifugation at 13000rpm for 1 min. Discard the flow-through in each case.
11. Spin the empty column at 13000rpm for 2min.

12. Place the GMiniSpin column in a clean 1.5mLmicrocentrifuge tube and add 30µL of nuclease-free water to the center of GMini Spin column. Let it stand for 5mins and centrifuge at 13000rpm for 1min. Collect the eluted product (flow-through) and store at 4°C.

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 at the bottom of the tube.

	Universal Primer Mix		*P. falciparum* Primer Mix		*P. vivax* Primer Mix		Control Primer Mix
	+	-	+	-	+	-	+
PCR Reagent Mix (2X)	12.5ul	12.5ul	12.5ul	12.5ul	12.5ul	12.5ul	12.5ul
Universal Primer Mix	1 ul	1 ul	-	-	-	-	-
P. falciparum Primer Mix			1 ul	1 ul			
P. vivax Primer Mix	-	-	-	-	1 ul	1 ul	-
Control Primer Mix	-	-	-	-	-	-	1 ul
Nuclease Free Water	6.5 ul	11.5 ul	6.5 ul	11.5 ul	6.5 ul	11.5 ul	6.5 ul

Set up a 25µL PCR reaction by adding the following constituents in a PCR tube:
Add the isolated DNA in following manner in '+' mark tube:

	Universal Primer Mix		*P. falciparum* Primer Mix		*P. vivax* Primer Mix		Control Primer Mix
	+	-	+	-	+	-	+
Isolated gDNA	5ul	-	5ul	-	5ul	-	5ul

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

PCR Protocol:

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

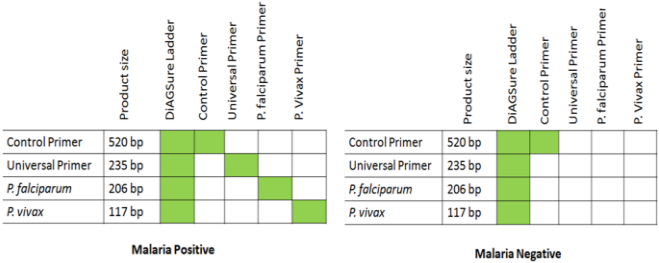
Agarose gel electrophoresis and gel visualization:

1. Cast a 2% agarose gel as follows:
   Weigh out 1g of agarose and add it to 50mL of 1X TAE buffer taken in a conical flask and heat it until the agarose gets dissolved completely. Cool the flask slightly and add 2µL of 10mg/mL Ethidium bromide solution and pour immediately into the gel casting tray. After gel solidification, place the gel in 1X TAE buffer for 15mins for equilibration.

2. Run 15 ul of PCR product along with 2 ul of 6x gel loading dye after loading it into appropriate wells of the gel placed in the gel running apparatus containing fresh 1X TAE buffer. Visualize the gel in a uv-trans illuminator or a gel documentation instrument.
3. Run 5 ul of DiAGSure DNA ladder.

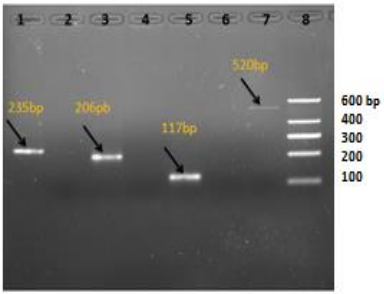
Results Interpretation:

Finally conclude your result as per following:



Here the Green color denotes the presence of band.

Fig 1. Representative gel image showing amplification of the Plasmodium pathogenic genes.



1	235 bp (*Plasmodium* genus)
2	235 bp NTC
3	206 bp (*P.falciparum*)
4	206 bp NTC
5	117 bp (*P.vivax*)
6	117 bp NTC
7	520 bp (internal control)
8	Standard marker

Sensitivity:

The kit can identify 10^6 copies of *Plasmodium* genomic DNA.

Quality Control:

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

Troubleshooting Guide:

1. *No amplification in internal control*

- Ensure that DNA has been properly isolated.
- Use freshly isolated DNA for amplification.

- Verify that all reagents are added to the PCR reaction in indicated amounts and proper PCR conditions have been maintained.
- It is advisable to store the reagents in aliquots for multiple uses.

2. *Amplification in negative control*

- Indicates that reagents have been contaminated. Repeat the reaction using a fresh aliquot.

3. *Variability among replicates*

- This can be due to manual pipetting error. In case of multiple replicates, prepare a master mix and aliquot it into replicate tubes.

4. *Clear gel with no bands*

- Ensure that EtBr has been added to the gel.
- Ensure mild cooling of the gel post dissolution of the agarose prior to EtBr addition.

Safety information:

The DiAGSure Malaria 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.