Leishmania sp.
PCR Determination and Detection Kit

Cat. No.: PR7937C
Quantity: 50 Reactions

Shipment: Wet Ice
Storage: -20 °C

Description:
This kit is designed for qualitative detection of Leishmania sp. kinetoplast DNA (kDNA) in the Human sample by the method of Polymerase Chain Reaction. Using this kit species of Leishmania Parasite could be determined by different PCR products size. CinnaGen Leishmania sp. PCR Determination and Detection Kit may be used in clinical medicine to detect Leishmania sp.kDNA.

Kit Contents:
The kit for 50 amplification reactions consists of:

1. 1X PCR MIX	**1000 µl**
2. Taq DNA polymerase	**15µl (5µ/µl)**
3- DNase Free, Deionized Sterile Water	**5 ml**
4. Mineral Oil	**2 ml**
5. Positive DNA Control	**50µl (1pg/µl)**

Sample preparation:

Performed in Pre-amplification, specimen and control preparation area.

DNA purification using **DNG ™-plus (Cat. No.: DN8117C)** is recommended.

For DNA purification from culture media centrifuge 1 ml of culture media (contains not more than 10^6 cell/ml of parasites) at 10000 RPM for 10 min. and decant media completely than follow the DNG ™ - Plus protocol.

Not: DNA from wound and biopsy samples needs Protease treatment and could be extracted using **CinnaGen DNP™ Kit (Cat. No.: DN8115C).**

PCR Protocol:

Performed in Pre-amplification,
Reagent preparation area.

1. Take out the kit and unfreeze the tubes, then put all the tubes on ice. Vortex and spin tubes before opening. The final volume of each PCR reaction will be 25 µl.
2. Label new 0.5 ml tubes for amplification reaction(s) for test(s), positive and negative control.
3. Add the following reagents for each tube on ice:

1x PCR MIX	**20 µl**
Taq-DNA polymerase	**0.3 µl**

● **NOTE:** To avoid contamination all reagents must be taken with separate clean tips!

4. Mix the mixture thoroughly by shaking and spin.
5. To each tube add one-drop (20-25 µl) **mineral oil.**
6. Add 5µl **DNA** (Use specified pipette for sampling of DNA).
7. Close tubes, spin the mixtures on microfuge for 3-5 sec.
8. Transfer the tubes to preheated thermocycler and start the program:
Cycling parameters:

95°C-180 sec		93°C-40 sec
63°C-30 sec	Follow by =>	63°C-40 sec
72°C-60 sec		72°C-60 sec
1 cycle		35 cycles

Result analysis:
Performed in Post-amplification area
Analyze 10 µl of amplified samples directly in a 2% agarose gel without adding loading buffer. The presence of 620 bp fragment indicates of L. major and 800 bp indicates of L. tropica. L.infantum also produces 800 bp fragment that should be determined according kind of sample and clinical history. For gel electrophoresis use of 100 bp Ladder (Cat. No. PR901644) is recommended.
For any further information about this Kit , please contact to: +98 912 135 0 829 Tel: +9826 36106485-6,
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