Leishmania tropica PCR Detection Kit

PD-LT001-01

100 Tests

Protocol

- **For Research Use Only.**

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Introduction

Leishmania is a genus of protozoa comprising parasites of worldwide distribution, several species of which are pathogenic for humans. *Leishmania tropica* is one of the causative agents of cutaneous leishmaniasis (CL), a disfiguring parasitic disease that recently was found to be viscerotropic. In urban areas it is transmitted from infected individuals by the bite of phlebotomine sand flies to naïve persons (anthroponotic CL). In rural areas animals are thought to be the reservoir, but the full life cycle is still under investigation (zoonotic CL). For many years *L. tropica* was either confused or merely grouped with *L. major* while Phlebotomus sergenti was the only proven vector.

Leishmania tropica is very heterogeneous, displaying serological, biochemical, and genetic heterogeneity. The genetic exchange is suggested to be the reason for high degree of heterogeneity in *L. tropica*. In recent years new foci have erupted, but few have been investigated. This review describes some of the history, recent findings, epidemiology, potential vectors, and the search for possible reservoir hosts besides man.

Principle of the PCR

By Polymerase Chain Reaction (PCR) method a specific sequence of DNA is amplified to increase the amount of the initial DNA in the reaction. Generally the reaction is carried out by DNA polymerase, nucleotides, and complementary primers that specific to target DNA sequence. Repeated cycles PCR steps exponentially increases the number of target DNA sequences.

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Kit specificity

***Leishmania tropica* PCR Detection Kit** has demonstrated high inclusivity for detection of *Leishmania tropica* strains. The primer and probe set of the kit has been designed with 100% homology to broadest number of different strains of *Leishmania tropica*. For more information, refer to **Appendix A**, "Specificity" on page 5.

Storage

Follow the guidelines below for storing BM *Leishmania tropica* PCR Kit:

- On receipt, store the all the reagents at –20 °C.
- Minimize freeze-thaw cycles.

Shelf life

The kit expires one year after shipment.

Kit Contents

Cap Color	Component Name	Volume (µL)
	**2X Master Mix, 1 Tube**	**600**
	**Hot Start Taq Polymerase, 1 Tube**	**60**
	**Positive Control- *Leishmania tropica* Template, 1 Tube**	**100**
	**PCR Grade Water, 2 Tube**	**1000**

Equipment and materials not included

- PCR thermal cycler Instrument
- Pipettors and Pipette tips, aerosol resistant
- Vortex and centrifuge
- Thin walled 1.5 ml PCR reaction tubes

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Prepare PCR

1. Create and set up a thermal cycling conditions specified in the following table.

Temperature	Time	Cycle
95°C	15 min	1
95°C	30 Sec.	37
56°C	30 Sec.	
72°C	40 Sec.	
72°C	7 min	1
4°C	-	-

2. Thaw all reagents completely.
3. Create the Master Mix Solution according to the following table.

Component	Volume
2X PCR Master Mix	6 µl
Hot Satart TaqPolymerase	0,6 µl
PCR Grade Water	13,4 µl
Total Volume	**20 µl**

4. Transfer 20 µl of Premix Solution into each well to be used, gently pipetting at the bottom of the well.
5. Transfer 5 µl of unknown sample into each sample well, gently pipetting up and down to mix the solution.
6. Transfer 5 µl of negative control (PCR Grade Water) into each negative-control well, gently pipetting up and down to mix the solution.
7. Transfer 5 µl of positive control into each positive-control well, gently pipetting up and down to mix the solution.

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Note: Use a new tip for each well.

8. Close the tubes or apply a cover film to the plate.
9. Make sure that the reagents are in the bottom of the wells

Gel electrophoresis

Agarose gel electrophoresis is employed to determine the presence or absence of PCR products and quantify the size (length of the DNA molecule) of the product for size separation of the PCR products. The size(s) of PCR products is determined by comparison with a DNA ladder. 10-12 µL of PCR product is loaded to 1.8X agarose gel and run the electrophoresis for about 90-120 minutes at 100 volt. Stain the gel to visualize the fragmented bands. The expected PCR product size for ***Leishmania tropica*** is **84** base pair.

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Appendix A

Specificity

Inclusivity of strains detected by ***Leishmania tropica*** **PCR Detection Kit:**

- Genbank accession numbers:

JX560482.1	FJ948465.1	AJ000301.1
JX560481.1	FJ948464.1	JX183382.1
JX560480.1	FJ948463.1	GQ920677.1
JX560479.1	FJ948462.1	GQ920676.1
JX560478.1	FJ948461.1	GQ920675.1
JX560477.1	FJ948460.1	GQ920674.1
JX560476.1	FJ948459.1	GQ920673.1
JX560475.1	FJ948458.1	FJ948455.1
JX560474.1	FJ948457.1	
JX560473.1	FJ948456.1	
JX560472.1	FJ948454.1	
JX560471.1	FJ948453.1	
JX560470.1	FJ948452.1	
JX560469.1	FJ948451.1	
JX560468.1	FJ948450.1	
JX560467.1	FJ948449.1	
JX560466.1	FJ948448.1	
JX560465.1	FJ948447.1	
JX560464.1	AJ300485.1	
JX104546.1	AJ000302.1	

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