Real-Time PCR *Leishmania tropica* Quantification Kit

PD-LT002-01

150 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 Real-Time 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. Repeated cycles PCR steps exponentially increases the number of target DNA sequences. In TaqMan method the probe has a reporter dye at one end and a fluorescence suppressing quencher on the other end. During PCR by hybridization of the probe to specific target sequence results in degradation of probe. The increase in fluorescence signal level is monitored as the amount of target sequence is amplified at each PCR cycle.

Kit specificity

Real-time PCR *Leishmania tropica* Quantification 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 strain number of different strains of *Leishmania tropica*. For more information, refer to **Appendix A**, “Specificity” on page 5.

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Storage

Follow the guidelines below for storing BM Real-time PCR *Leishmania tropica.* Quantification Kit:

- On receipt, store the all the reagents at –20 °C.
- Protect from light. Excessive exposure to light may affect the fluorescent probes.
- Minimize freeze-thaw cycles.

Shelf life

The kit expires one year after shipment.

Kit Contents

Cap Color	Component Name	Volume (µL)
	BM 5X qPCR MasterMix *, 1 Tube	**600**
	***Leishmania tropica* Primer/Probe Assay Mix 1 Tube**	**240**
	Positive Control- *Leishmania tropica*. Template, 1 Tube	**200**

*Contains HotStarTaq® DNA Polymerase, qPCR Buffer, dNTP mix (dATP, dCTP, dGTP, and dTTP), and ROX passive reference dye.

Equipment and materials not included

- Real-Time PCR Instrument
- 96-Well Optical Reaction Plate, Optical Caps, (8 caps/strip)
- Pipettors and Pipette tips, aerosol resistant
- Vortex and centrifuge
- Thin walled 1.5 ml PCR reaction tubes
- RNase-free, sterile-filtered water

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Preparation of Positive Control standards

1. Transfer 900 µl Nuclease Free Water into 5 tubes ladled from 2 to 6
2. Transfer 100 µl of Positive Control- *Leishmania tropica* Template to tube 2 and mix by votexing
3. Transfer 100 µl form tube 2 to tube 3 and mix by votexing
    - Repeat steps 2 and 3 to prepare dilution series

Standard Sample	Copy Number
Positive Control	2×10^5 per µl
Tube 2	2×10^4 per µl
Tube 3	2×10^3 per µl
Tube 4	2×10^2 per µl
Tube 5	20 per µl
Tube 6	2 µl

Prepare PCR

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

Temperature	Time	Cycle
95°C	15 min	1
95°C	15 Sec.	40
60°C	1 min	

2. Check the JOE(~550 nm) channel for detection of target specific signal
3. Thaw all reagents completely.
4. Create the MasterMix Solution according to the following table.

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Component	Volume
5X qPCR Master Mix	4 µl
Leishmania tropica Primer/Probe Assay Mix	1,6 µl
PCR Grade Water	9,4 µl
Total Volume	**15 µl**

5. Transfer 15 µl of Premix Solution into each well to be used, gently pipetting at the bottom of the well.
6. Transfer 5 µl of unknown sample into each sample well, gently pipetting up and down to mix the solution.
7. Transfer 5 µl of negative control (PCR Grade Water) into each negative-control well, gently pipetting up and down to mix the solution.
8. Transfer 5 µl of positive control into each positive-control well, gently pipetting up and down to mix the solution.

   Note: Use a new tip for each well.
9. Close the tubes or apply an optical cover to the plate.
10. Make sure that the reagents are in the bottom of the wells. If available, use a centrifuge with a plate adapter to briefly centrifuge the plate.

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Appendix A

Specificity

Inclusivity of strains detected by **Real-time PCR *Leishmania tropica* Quantification 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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