LightCycler Real-Time PCR

Leishmania donovani complex LCSet

Guidelines for the proper use of the reagents (for use on LightCycler 1.x / 2.0)

Assay description:

The LightCycler – *Leishmania donovani* complex (Leish.don.) LCSet is specifically adapted for PCR in glass capillaries using the LightCycler Instrument and Hybridization Probes for identification of *L. infantum, L. donovani and L. chagasi* in research samples.

A conserved genomic fragment of the *L. infantum, L. donovani and L. chagasi* kinetoplast minicircle is amplified with specific primers. The amplicon is detected by fluorescence using a specific pair of Hybridization Probes labeled with LightCycler-Red 640.
The Hybridization Probes consist of two different oligonucleotides that hybridize to an internal sequence of the amplified fragment during the annealing phase of the PCR cycle. One probe is labeled at the 5'-end with LightCycler-Red 640 and, to avoid extension, modified at the 3'-end by phosphorylation. The other probe is labeled at the 3'-end with fluorescein. Only after hybridization to the template DNA, the two probes come in close proximity, resulting in fluorescence resonance energy transfer (FRET) between the two fluorophores. During FRET, fluorescein, the donor fluorophore, is excited by the light source of the LightCycler Instrument, and part of the excitation energy is transferred to LightCycler - Red 640, the acceptor fluorophore. The LightCycler Instrument then measures the emitted fluorescence of LightCycler-Red 640.

Note: The described performance of the assay can be guaranteed only when used on the LightCycler Instrument.

This set was developed for use in life science research only.
It must not be used for diagnostic applications.

Assay contents:

Leish.don. P&P (Primer and Hybridization Probes)	96	Reactions
Leish.don. Positive Control	9	Row dilution

Sample preparation:

Perform nucleic acid purification from biological samples (peripheral blood, bone marrow or lymph node biopsies) using the High Pure PCR Template Preparation Kit (Cat. No. 11 796 828 001), **use 50 µl of Elution buffer.**

Reagents preparation:

Spin both tubes for 30 sec at max speed (about 10,000 RPM), as oligonucleotides are very light and can fly away when you open the tubes!

Dissolve Leish.don. P&P with 103 µl "PCR grade" H_2O.

Note: Primers and probes are stable if stored in the dark at 4°C for 1 month; if you use the reagents over a longer period of time, make aliquots and store frozen.

Repeated cycles of freezing and thawing are strongly discouraged.

Positive control preparation:

Dissolve the Leish.don. Positive Control with 100 µl "PCR grade" H_2O and proceed with serial dilutions as described in the table.

Stock solution	10^{-1}	10^{-2}	10^{-3}
10^{0}	10 µl		
	90 µl water		
	10^{-1}	10 µl	
		90 µl water	
		10^{-2}	10 µl
			90 µl water
		Working solution	10^{-3}

Use 5 µl Ctr. Pos.
Dispose the diluted reagents after use.

Note: Positive control stock solution is stable 1 week if stored in the dark at 4°C;
We suggest to make aliquots and store frozen.

We recommend programming the LightCycler instrument before the preparation of solutions and samples (see next page).

Master Mix

Amplification mix	Final Volume / Sample	
FastStart DNA Master^{plus} Hybridization Probes	4.00 µl	Roche 03 515 575 001
Leish. don. P&P	1.00 µl	Included
DNA	5.00 µl	
H_2O	10.00 µl	

Always run a positive and a negative control.

PCR Protocol						
Display Mode	**F2 / F1 640 / 530**	**Color Compensation Off**				
Cycles	**1**	**Analysis mode**		**None**		
Taq Activation	**Step1**	**Step2**	**Step3**	**Step4**	**Step5**	
Target Temp. (°C)	95					
Incubation time (sec)	480					
Temp. Transition Rate (°C/sec)	20.0					
Secondary target temp. (°C)	0					
Step size (°C)	0.0					
Step delay (Cycles)	0					
Acquisition mode	None					
Cycles	**55**	**Analysis mode**		**Quantification**		
Amplification	**Step1**	**Step2**	**Step3**	**Step4**	**Step5**	
Target Temp. (°C)	95	60	72			
Incubation time (sec)	5	5	4			
Temp. Transition Rate (°C/sec)	20.0	20.0	20.0			
Secondary target temp. (°C)	0	0	0			
Step size (°C)	0.0	0.0	0.0			
Step delay (Cycles)	0	0	0			
Acquisition mode	None	Single	None			
Cycles	**1**	**Analysis mode**		**Melting Curve**		
Melting curve and cooling	**Step1**	**Step2**	**Step3**	**Step4**	**Step5**	**Step6**
Target Temp. (°C)	72	95	40	85	40	
Incubation time (sec)	30	20	30	1	5	
Temp. Transition Rate (°C/sec)	20.0	20.0	20.0	0.5	20.0	
Secondary target temp. (°C)	0	0	0	0	0	
Step size (°C)	0.0	0.0	0.0	0.0	0.0	
Step delay (Cycles)	0	0	0	0	0	
Acquisition mode	None	None	None	Step	None	

Positive Control amplification

The amplification of 5 µl of the Positive Control should have a crossing point before the 30th cycle.

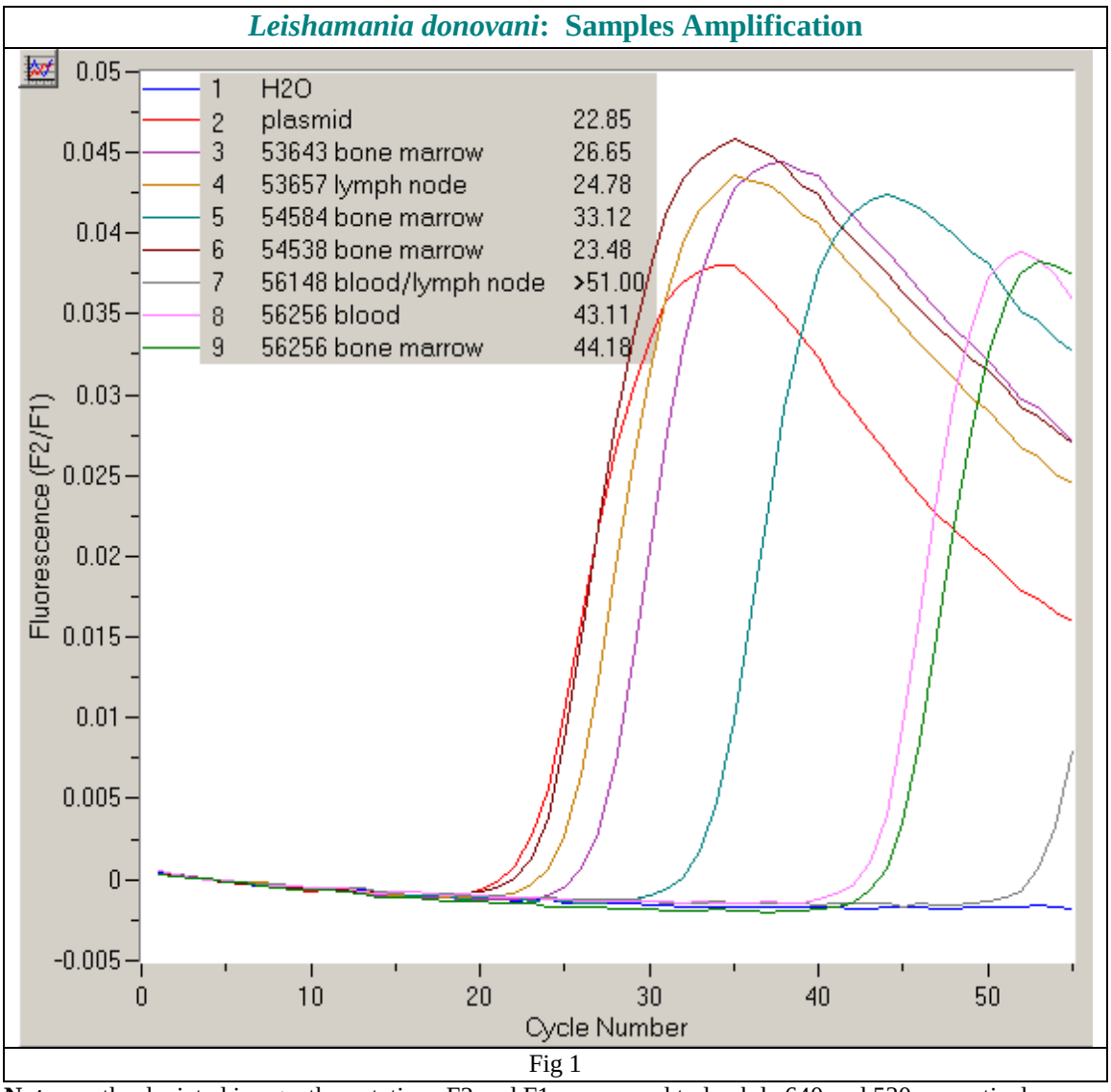

Fig 1

Note: on the depicted image, the notations F2 and F1 correspond to lambda 640 and 530 respectively.

Specific amplification for *Leishmania donovani* complex is depicted.
Capillary number 1 is the Negative Control.
Capillary number 2 is the Positive Control.
Capillary numbers 3 to 9 are positive samples for *Leishmania donovani* complex.