LightMix® Modular Schistosoma spp.

580

Cat.-No. 58-0639-96 Roche SAP n° 07 225 270 001

Kit with reagents for 96 PCR reactions 20 µl for detection of *Schistosoma* [lyophilized]

1. Content, Storage and Expiry

1 Vial red cap 96 reactions Schistosoma (lyophilized)
1 Vial black cap Positive Control (≈ Cp 30), lyophilized

Storage at Arrival:

Store cooled or at ambient temperature
Do not freeze the lyophilized reagents.

- Kits are stable for one year after production (store 4°C to 25°C in the dark). See lot-specific expiry date.
- Reconstituted reagents are stable for two weeks if stored protected from light and cooled (2°C to 8°C).
- Dissolved reagent can be stored long-term if frozen (-15°C to -25°C). Avoid multiple freeze-thaw cycles.
- Reconstituted positive controls must be stored frozen. Minimize multiple freeze-thaw cycles.

2. Additional Reagents required

LightCycler® Multiplex DNA Master	Cat.-No. 07 339 585 001
or Roche LightCycler® 480 Probes Master (no instructions included)	Cat.-No. 04 707 494 001

3. Introduction

Trematodes of the genus Schistosoma cause bilharziosis (schistosomiasis), a chronic illness damaging internal organs. Acute schistosomiasis may occur weeks after the initial infection (Katayama's fever). *Schistosoma mansoni* and *S. intercalatum* (in Asia *S. japonicum* and *S. mekongi*) cause the intestinal schistosomiasis while *S. haematobium* causes the urinary schistosomiasis. There are further species infecting different animals. Transmission is through contact to water containing freshwater snails which carry the parasite, especially in Africa, Asia and South America.

4. Description

A 78 bp long fragment from the ribosomal RNA spacer ITS-2 gene is amplified with specific primers and detected with a R6G labeled hydrolysis probe (580 channel).

5. Specification

This assay detects 10 target copies or less per reaction (plasmid DNA dilution).

6. Sample Material and Extraction

Typical samples are urine or from feces.
For extraction protocols see Roche MagNA Pure or Roche manual kit instructions.

7. Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which do not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

This product is not hazardous, toxic, or IATA-restricted. This product is not from human, animal or plant origin. Product contains synthetic oligonucleotide primers and probes.

8. Instructions for Use

When run in combination with assays with other fluorophores (channels), a Color Compensation file must be applied. To generate a Color Compensation file see instructions in the ***Roche 06296971001 Universal Color Compensation Hexaplex*** Instructions For Use.

8.1. Programming Roche 480 Instruments

See the Instrument operator's manual for details. Start programming before preparing the solutions. The protocol consists of three program steps:

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification
- 3: Cooling: cooling the instrument

Detection Format 580 Channel	**Set Quant Factor 10, Max Integration time 1 sec**
LightCycler® 480 Instrument:	523-568
LightCycler® 480 II Instrument:	533-580
cobas z 480 Analyzer (open channel):	540-580

Program Step:	*RT Step**	***Denaturation***	***Cycling***			***Cooling***
Parameter						
Analysis Mode	*None*	None	Quantification mode			None
Cycles	*1*	1	45			1
Target [°C]	55	95	95	60	72	40
Hold [hh:mm:ss]	00:05:00	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30
Ramp Rate [°C/s] **96**	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] **384**	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	**Single**	None	None

* *optional use if combining with 1-Step RT-PCR*

Table 1

8.2. Experimental Protocol

- **Sample material:** Use aqueous nucleic acid preparations (e.g. 'High Pure PCR Template Preparation Kit').
- **Negative control:** Always run at least one no-template control (NTC) - replace the template NA with water.
- **Positive control:** Run a positive control - replace the template NA with the provided Positive Control.

For an increased sensitivity use 10 µl nucleic acid per 20 µl reaction, for sample types were inhibition may occur e.g. Fecal sample extracts, use 5 µl. For 10 µl reactions in 384 well plates use 5 µl /2.5 µl.

8.2.1. Preparation of Parameter-Specific Reagents (PSR, 96 reactions):

The reagent vial with a **red** cap contains primers and probe to run 96+ LightCycler® reactions.

Check for the colored pellet, then **add 50 µl** PCR-grade water, mix (vortex) and spin down.
For robotic pipetting the volume can be extended to 55 µl (signals will decrease by 10-20%).

►**Use 0.5 µl** reagent for a 20 µl PCR reaction.

8.2.2. Preparation of the Positive Control

Add 160 µl RNase/DNase-free 10 mM Tris buffer pH 8 - 8.5 to the vial with the **black** cap, if using 10 µl sample volume add **320 µl**. Mix by pipetting up and down 10 times. If vortexing spin down to collect the solution. Store dissolved controls frozen. Use of Tris increases the stability in solution.

Notes: Opening this vial may cause contamination of the workspace. Pulse spin vial prior to opening.

►**Use 5 µl** positive control (≈ Cp 30) for a 20 µl PCR reaction (10 µl if using 10 µl sample volume).

8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve, prepare in a cooled tube:

For use with the Roche LightCycler® Multiplex DNA Master		
for 5 µl extract	**Component**	**10 µl extract**
10.5 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**Roche Master** (see Roche manual)	4.0 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 µl**

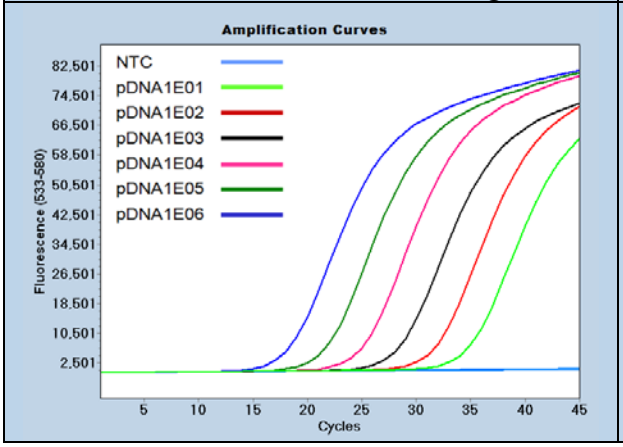
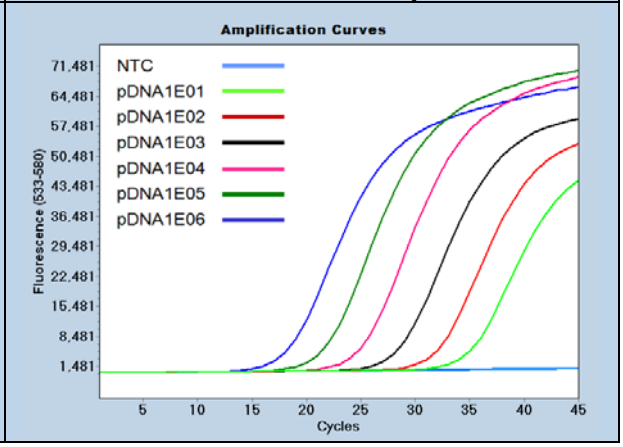
Table 2

Mix gently, spin down and **transfer 15 µl (10 µl)** per well.

Add 5 µl (10 µl) of sample or control to each well for a final reaction volume of 20 µl. Seal plate and centrifuge.

Start run

9. Typical Results (Data from LightCycler® 480 II system)

Channel 580 : Schistosoma– Single PCR	Data obtained in Multiplex PCR
	

Dilution row 1E6 to 10 copies / reaction

Figure 1

10. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation. We recommend using the Second Derivative Maximum method (Automated (F" max). View results in the 580 channel. The negative control (NTC) must show no signal.

Channel 580 (sample)	Channel 660 Control Reaction	Channel 580 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 37 +	Not relevant	**Negative**	**Schistosoma Positive**
No amplification	**Not detectable**	Not relevant	**PCR failure Repeat**
Amplification signal	Not relevant	**Positive**	**Contamination Repeat**

Note: cobas z 480 Analyzer signal levels are ~ 50% as compared to LightCycler® 480 II results.
+ Recommendation: Define the cut-off 2-4 cycles higher than observed Cp value for 10 copies.

11. References

Novel Automated Biomarker Discovery Workflow for Urinary Peptidomics. Balog et al., ClinChem. (2008)

12. Multiplex PCR Compatibility

This assay can be combined with other assays up to 6plex reactions including an internal control (IC) or a spiked extraction control (for example PhHV) as depicted below :

Parasite Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660	480 II	z 480	LC96	LC2.0	Nano
		Schistosoma			PhHV	X	X	X		X
N. americanus		Schistosoma				X	X	X		
		Schistosoma		A.doudenale						
N. americanus		Schistosoma		A.doudenale						

Table 3

13. Version History

V140404	Release version	2014-04-14
V140909	Editorial changes	2014-09-09
V151001	2015 protocol Multiplex Master, 5/10 µl extract and 60°C acquisition	2015-10-01
V160313	1. Storage of controls, 8.2.2 buffer, 8.2.3 wording	2016-06-10
V161212	Reverse primer and probe shortened to improve multiplex compatibility	2016-12-21
V190123	Editorial changes 8.2.2 Use Tris buffer	2019-02-27

Certificate of Analysis (CoA)

Lot n°
Expiry :

TIB MOLBIOL

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range				27-30			
Measured							
Signal level				60-75			
Measured							
Negatives	10/10						✓

***Note**:* Cp (crossing point) values collected with pDNA (single target PCR). Fluorescence (FL) levels depend on instrument settings and may vary. The Cp values will vary from instrument to instrument by up to 2 cycles, while the distance between two dilution steps should be relative constant (ΔCp).

QC Acceptance Date: YYYYMMDD

We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.

Name(s) :

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