



Instructions for life science research use only. Not tested for use in diagnostic procedures. For *in vitro* use only.



Instructions For Use

## LightMix<sup>®</sup> Modular *Ancylostoma duodenale*

640

Cat.-No. 64-0710-96

Roche SAP n° 00 000 000 000

Kit with reagents for 96 PCR reactions 20 µl for detection of *A. duodenale* [lyophilized]

### 1. Content Storage and Expiry

#### Storage at Arrival:

- 1 Vial blue cap 96 reactions *A. duodenale* (lyophilized)
- 1 Vial black cap Positive Control (32,000 copies, lyophilized)

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

- Lyophilized kits are stable for at least 6 months (4°C to 25°C in the dark). See lot-specific expiry date.
- Dissolved reagents are stable for at least 2 weeks if stored protected from light and cooled (4°C).
- Dissolved reagents can be stored long-term at -20°C (within expiry). Avoid multiple freeze-thaw cycles.
- Dissolved positive controls must be stored at -20°C. Avoid multiple freeze-thaw cycles.

### 2. Additional Reagents required

LightCycler<sup>®</sup> Multiplex DNA Master  
or Roche LightCycler<sup>®</sup> 480 Probes Master (no instructions included)

Cat.-No. 07 339 585 001  
Cat.-No. 04 707 494 001

### 3. Introduction

Infectious diarrhea can be caused by viruses, bacteria or parasites. *Ancylostoma duodenale* is a parasitic nematode worm and commonly known as the Old World hookworm. It lives in the small intestine of hosts such as humans, cats and dogs and it causes the hookworm infection. *Ancylostoma duodenale* is abundant throughout the world.

### 4. Description

A 90 bp long fragment from the ITS2 gene is amplified with specific primers and detected with a LC640 labeled hydrolysis probe. This assay will also detect *A. ceylanicum*.

### 5. Specification

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

### 6. Sample Material and Extraction

Typical samples are from feces or eventually rectal swabs. See ModularDx **Extraction Protocols**.

### 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).

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



## 8. Instructions for Use

- Instrument programming see document
- Color Compensation see instructions in
- Pipetting instructions multiplex PCR see

**ModularDx Programming**

**40-0320 Universal Color Compensation Hexaplex**

**ModularDx Multiplex**

### 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 640 Channel

LightCycler® 480 Instrument:

LightCycler® 480 II Instrument:

cobas z 480 Analyzer (open channel):

**Set Quant Factor 10, Max Integration time 3 sec**

523-640

533-640

540-645

Program Step:	RT Step*	Denaturation	Cycling			Cooling
<b>Parameter</b>						
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] <b>96</b>	4.4	4.4	4.4	2.2	4.4	1.5
Ramp Rate [°C/s] <b>384</b>	4.6	4.6	4.6	2.4	4.6	2.0
Acquisition Mode	None	None	None	Single	None	None

\* optional to combine 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 sample per 20 µl reaction, in case that inhibition is likely to occur, e.g. extracts obtained from fecal samples, 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):

One reagent vial with a **blue** cap contains all primers and probe to run 96+ LightCycler® reactions.

**Add 50 µl** PCR-grade water to each reagent vial, mix the solution (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 Tris buffer or water to the vial with the **black** cap, for 10 µl sample add **320 µl**. Mix the solution by pipetting up and down 10 times. If vortexing spin down to collect the solution.

**Notes:** Opening of this vial may cause contaminations of the work-space (aerosol). Use of Tris buffer pH 8.0-8.5 increases the long-term stability in solution. Store dissolved controls frozen.

► **Use 5 µl** positive control (≈ 1,000 copies) for a 20 µl PCR reaction (or 10 µl if using 10 µl sample).

### 8.2.3. Preparation of the Reaction Mix

Multiply volumes by the number of reactions plus controls and one reserve and 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	<b>Water</b> , PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	<b>Reagent</b> mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	<b>Roche Master</b> (see Roche manual)	4.0 µl
<b>15.0 µl</b>	<b>Volume of Reaction Mix</b>	<b>10.0 µl</b>

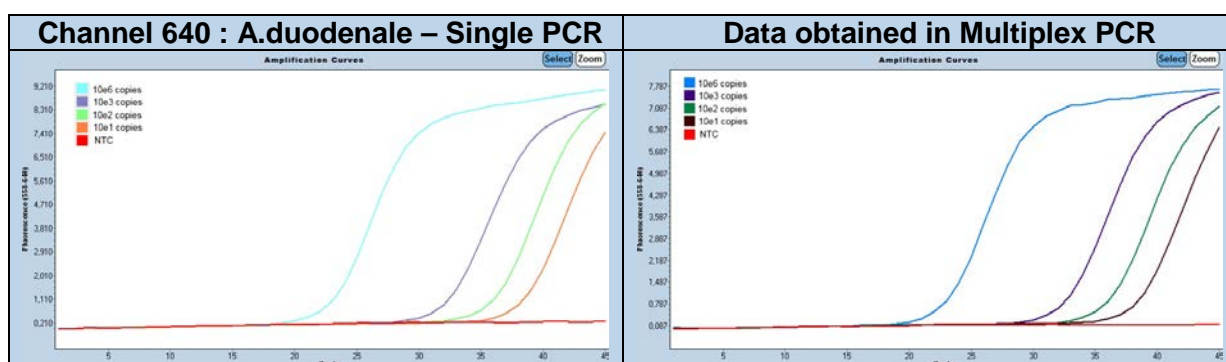
Table 2

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)



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 640 channel. The negative control (NTC) must show no signal.

Channel 640 (sample)	Channel 660 Control Reaction	Channel 640 NTC Control	Result
No amplification	Detectable	Negative	Not detectable
<b>Amplification Cp &lt; 37<sup>+</sup></b>	Not relevant	Negative	<b>A.duodenale Positive</b>
<b>No amplification</b>	<b>Not detectable</b>	Not relevant	<b>PCR failure Repeat</b>
<b>Amplification signal</b>	Not relevant	<b>Positive</b>	<b>Contamination Repeat</b>

**Notes:** cobas z 480 Analyzer signal levels are about 50% compared to LightCycler® 480 II results.

+ Recommendation : Define the cut-off 2-4 cycles higher than observed for 10 copies

### 11. References

Simultaneous Detection and Quantification of Ancylostoma duodenale, Necator americanus, and Oesophagostomum bifurcum in Fecal Samples Using Multiplex Real-Time PCR. Verweij, Brienen, Ziem, Yelifari, Polderman, and Van Lieshout. Am. J. Trop. Med. Hyg., 77(4), 2007, pp. 685–690.

## 12. Multiplex PCR Compatibility (Gastro Panel)

This A.duodenale 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 :

**Gastro Multiplex PCR and Instrument Compatibility**  
Color Compensation 40-0320 is mandatory for Multiplex PCR

500	530	580	610	640	660
	Cyclospora	S. stercoralis	A.lumbricoides	A.duodenale	PhHV
N.americanus	Cyclospora	S. stercoralis	A.lumbricoides	A.duodenale	
E.vermicularis	Cyclospora	S. stercoralis	A.lumbricoides	A.duodenale	
				A.duodenale	


480 II	z 480	LC96	LC2.0	Nano
X	X			
X	X			
X				
X				

Table 3

This kit shares the 640 channel with 64-0709-96 Hymenolepis nana.

## 13. Version History

V151001	Release version	2016-01-26
V160313	1. Storage of controls, 8.2.2 buffer, 8.2.3 wording, 12. Combinations	2016-07-10

<b>Certificate of Analysis (CoA)</b>							
Lot n° Expiry :							
<b>Dilution</b>	1E6	1E5	1E4	PC	1E2	1E1	<b>passed</b>
<b>Cp range</b>							
<b>Measured</b>							
<b>Signal level</b>							
<b>Measured</b>							
<b>Negatives</b>	10/10						✓
<b>Note:</b> 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 ( $\Delta$ Cp).							
<b>QC Acceptance Date:</b>				YYYYMMDD			
We, the undersigned, certify that the product designated above has been obtained in accordance with the rules of production and quality control.							
<b>Name(s) :</b>							

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