Instructions For Use

LightMix® Modular *Chlamydia trachomatis*

500

Cat.-No. 50-0760-96 Roche SAP n° 08 984 549 001

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

1. Content Storage and Expiry

Storage at Arrival:

1 Vial orange cap 96 reactions *C.trachomatis* (lyophilized)
1 Vial black cap Positive Control (≈ Cp 30), lyophilized

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

3. Introduction

Chlamydia trachomatis is an obligate intracellular parasite that exclusively infects humans, and is the most prevalent sexually transmitted pathogen. Infected individuals show oftenno symptoms. In women CT may cause vaginal discharge, burning during urination, or bleeding after sexual intercourse. In men, non-gonococcal urethritis is the main symptom.

4. Description

This kit detects two targets: A 64 bp long fragment from the cryptic plasmid is amplified with specific primers and detected with a Cyan500 labeled hydrolysis probe. A 137 bp fragment of the MOMP gene is amplified with specific primers.and detected with a Cyan500 labeled hydrolysis probe.

5. Specification

This assay detects 10 genome equivalent copies or less per reaction (lot release on pDNA dilution). Sensitivity determined in multiplex PCR was approx 2 bacteria per PCR.

6. Sample Material and Extraction

Typical sample type are vaginal swabs or urine.
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 500 Channel	**Set Quant Factor 10, Max Integration time 1 sec**
LightCycler® 480 Instrument:	450-500
LightCycler® 480 II Instrument:	440-488
cobas z 480 Analyzer (open channel):	No filter combination for Cyan500 (opt. use FAM channel)

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 an orange cap contains the 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:

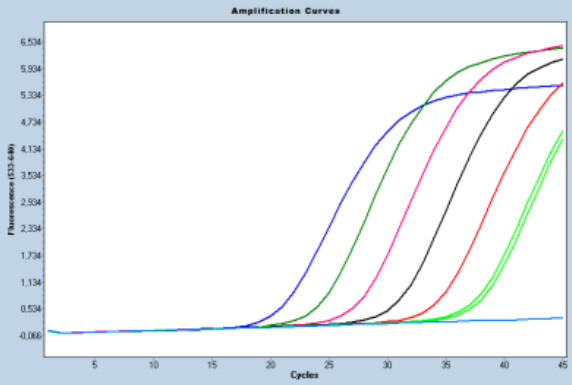
For use with the Roche LightCycler® Multiplex DNA Master		
for 5 µl extract	**Component**	**10 µl extract**
10.5 µl	**Water**, PCR-grade (colorless cap, provided with the Roche Master kit)	5.5 µl
0.5 µl	**Reagent** mix (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction 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 500 : CT – 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 500 channel. The negative control (NTC) must show no signal.

Channel 500 (sample)	Channel 660 Control Reaction	Channel 500 NTC Control	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 39+	**Not relevant**	**Negative**	**C.trachomatis Positive**
No amplification	**Not detectable**	**Not relevant**	**PCR failure Repeat**
Amplification signal	**Not relevant**	**Positive**	**Contamination Repeat**

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

11. References

Detection of C. trachomatis in urogenital specimens by polymerase chain reaction. Näher et al., (1991)

Comparison of three in-house multiplex PCR assays for the detection of Neisseria gonorrhoeae and Chlamydia trachomatis using real-time and conventional detection methodologies. Whiley Pathology. 2005

12. Multiplex PCR Compatibility (STI)

This kit 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:

STI 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
Chlamydia						X	X			
Chlamydia	M.genital	T.vaginalis	M.hominis	N.NG gyrA	PhHV	X				
Chlamydia	M.genital	T.vaginalis	NG opaD	Ureaplasma		X				
Chlamydia	M.genital	T.vaginalis	NG opaD	T.pallidum		X				
Chlamydia	M.genital	LGV	NG opaD	T.pallidum		X				
Chlamydia	M.genital	LGV	NG opaD	H.ducreyi		X				
Chlamydia	M.genital	LGV	NG opaD	Tp + H.duc		X				

Table 3

13. Version History

V180909	Release version	2018-09-30
V190123	8.2.1. Check for the pellet	2019-05-29

Certificate of Analysis (CoA)

Lot n°
Expiry :

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Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp range	18-20	21-23	24-27	28-30	31-33	34-36	
Measured							✓
Signal level				80-120			
Measured							✓
Negatives	10/10						✓

***Note**:* Fluorescence (FL) levels depend on instrument settings and may vary. The crossing point (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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