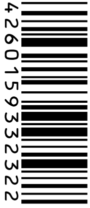

Instructions for life science research use only. Not tested for use in diagnostic procedures. For *in vitro* use only.

Instructions For Use

LightMix® Modular *Trichomonas vaginalis* RepeatDNA 580

Cat.-No. 58-0669-96 Roche SAP n° 10 171 987 001

Kit with reagents for 96 PCR reactions 20 µl for detection of *Trichomonas vaginalis DNA.*

1. Content, Storage and Expiry

1 Vial red cap 96 reactions *T. vaginalis* (dried)
1 Vial black cap Positive Control (dried)d

Storage at Arrival:

Store cooled or at ambient temperature
Do not freeze the dry 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 Roche Cat.-No. 07 339 585 001

3. Introduction

Trichomonas vaginalis (*T. vaginalis*) is a sexual transmitted protozoan parasite causing trichomoniasis. About half of the infected individuals stay asymptotic. In men the infection can cause urethritis while women develop vaginitis and have an increased risk for HIV-1 infections; for pregnant women preterm delivery has been reported. In contrast to *C. trachomatis, T. vaginalis* is found more in elderly people. The common diagnosis by culture has a sensitivity of only 75%-95%.

4. Description

A 106 bp long fragment from the repeated DNA target is amplified with specific primers and detected with a R6G labeled hydrolysis probe.

5. Specification

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

6. Sample Material and Extraction

Typical sample types are vaginal swabs or urine.
For extraction protocols see Roche MagNA Pure or Roche manual kit instructions.

7. Instructions for Use

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

7.1. Programming Roche 480 Instruments

For use with LightCycler® 480 Instruments and cobas z 480 Analyzer, software 1.5 and higher. See the Instrument operator's manual for details. Program the instrumentation prior to reagent preparation.

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

Table 1: Cycling condition programming

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 in the same run*

7.2. Experimental Protocol

- **Sample material:** Use aqueous nucleic acid (NA) 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.

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

The reagent vial with a **red** 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.

7.2.2. 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 for a 20 µl PCR reaction (10 µl if using 10 µl sample volume).

7.2.3. Preparation of the Reaction Mix

In a cooled tube, prepare the reaction mix by multiplying the single reaction volumes by the number of reactions including the controls plus one additional reaction; the smallest recommended pipetting volume is 1 µl.

Table 2: Pipetting instructions for one reaction mix.

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	**PSR** (parameter specific reagents containing primers and probes)	0.5 µl
--	Control Reaction and additional assays (Multiplex PCR)	--
4.0 µl	**LightCycler® Multiplex DNA Master** (see Roche manual)	4.0 µl
15.0 µl	**Volume of Reaction Mix**	**10.0 µl**

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

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

Start run

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

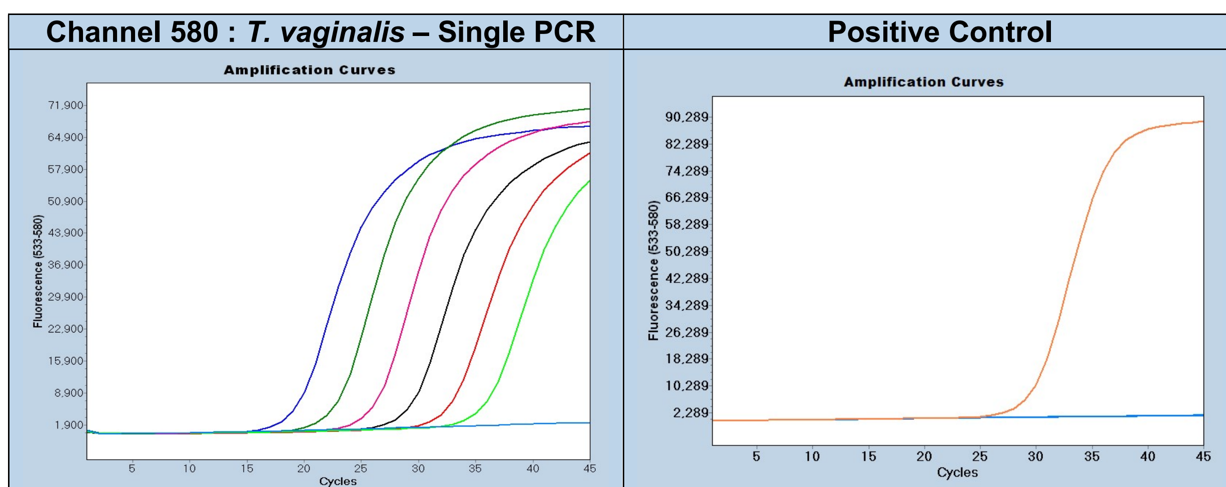

Figure 1: Results for dilution row 1E6 to 1E1 copies plasmid DNA and the Positive Control.

9. 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 R6G channel. The NTC must show no signal.

Table 3: Result analysis for individual channels.

Channel 580 (sample)	Channel 660 Control Reaction	Channel 580 NTC	Result
No amplification	**Detectable**	**Negative**	**Not detectable**
Amplification Cp < 38*	**Not relevant**	**Negative**	***T.vaginalis* 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 (see CoA).

10. References

Trichomonas vaginalis: repeated DNA target for highly sensitive and specific polymerase chain reaction diagnosis. Kengne P, Veas F, Vidal N, Rey JL, Cuny G. Cell Mol Biol (Noisy-le-grand) 1994)

11. Multiplex PCR Compatibility (STI Panel)

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:

Table 4: Optional combinations with other TIB Molbiol Modular products in a multiplex PCR

STI Multiplex PCR and Instrument Compatibility

Color Compensation 40-0320 is mandatory for Multiplex PCR

<table>
<tr><th>500</th><th>530</th><th>580</th><th>610</th><th>640</th><th>660</th><th>480 I</th><th>480 II</th><th>z 480</th><th>LC96</th><th></th></tr>
<tr><td></td><td></td><td>T. vaginalis</td><td>control</td><td></td><td></td><td>X</td><td>X</td><td>X</td><td>X</td><td></td></tr>
<tr><td></td><td>M.gen</td><td>T. vaginalis</td><td>M. hominis</td><td></td><td rowspan="5">PhHV</td><td>X</td><td>X</td><td>X</td><td>X</td><td></td></tr>
<tr><td></td><td>M.gen</td><td>T. vaginalis</td><td>M. hominis</td><td>NG gyrA</td><td>X</td><td>X</td><td>X</td><td></td><td></td></tr>
<tr><td></td><td>M.gen</td><td>T. vaginalis</td><td>NG opaD</td><td>Ureaplasma</td><td>X</td><td>X</td><td>X</td><td></td><td></td></tr>
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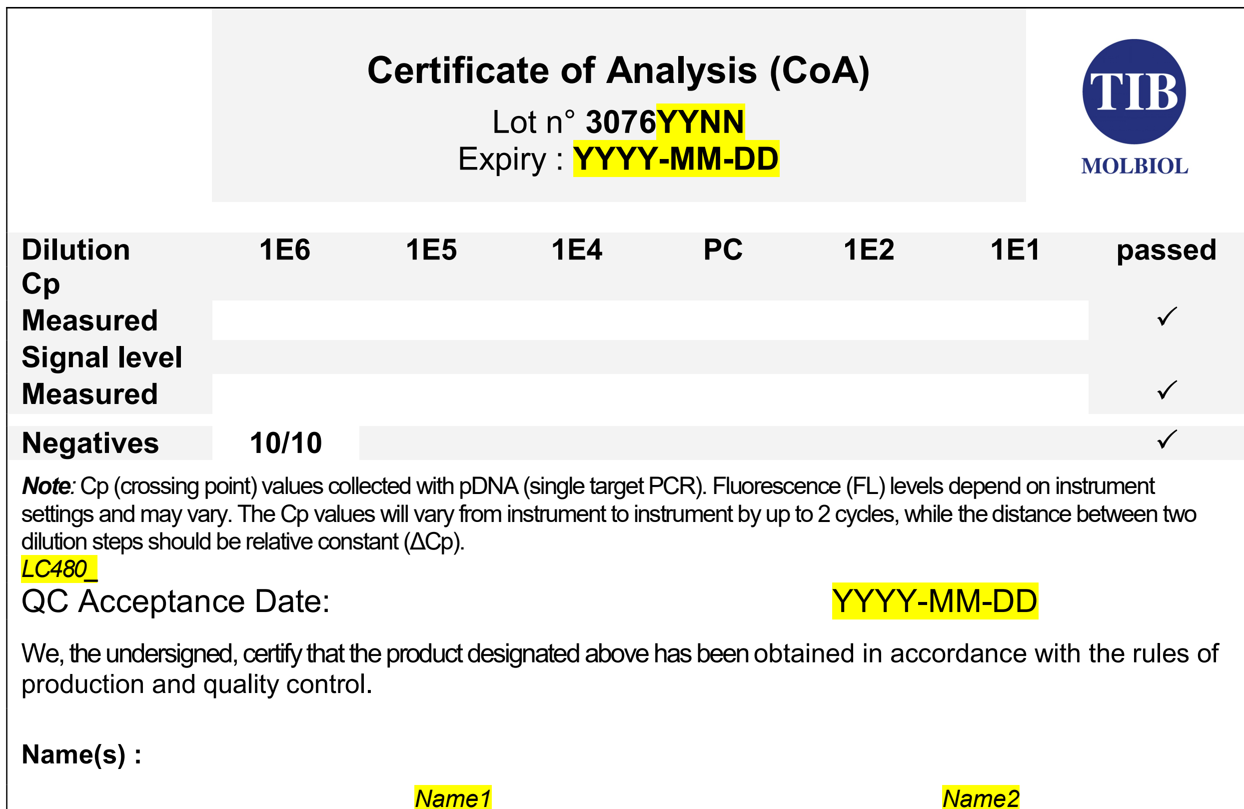
</table>

12. Version History

V180909	Cp range updated	2018-11-03
V190123	Editorial changes, 8.2.2 Use Tris buffer	2019-01-29
V231004	Roche SAP number included, chapters renumbered, editorial changes	2023-10-04



Certificate of Analysis (CoA)

Lot n° **3076YYNN**

Expiry : **YYYY-MM-DD**

TIB MOLBIOL

Dilution	1E6	1E5	1E4	PC	1E2	1E1	passed
Cp Measured							✓
Signal level 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).

*LC480_*

QC Acceptance Date: YYYY-MM-DD

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

Name(s) :

Name1 *Name2*

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