



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



530

Instructions For Use

LightMix® Modular *M.genitalium* + Macrolide

Cat.-No. 53-0665-96

Roche SAP n° 07 696 108 001

Kit with dried reagents for 96 PCR reactions 20 µl for detection of *M. genitalium* + Macrolide.

1. Content, Storage and Expiry

- 1 Vial yellow cap 96 reactions *M.gen+Macro* (dried)
- 1 Vial black cap Positive Control (dried)

- 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.

Storage at Arrival:

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

2. Additional Reagents required

LightCycler® Multiplex DNA Master

Roche Cat.-No. 07 339 585 001

3. Introduction

Mycoplasma genitalium (*M. genitalium*) is a small bacterium with the ability to cause urethritis in men and urethritis, cervicitis, and pelvic inflammatory disease in women. Recently resistant types of *M. genitalium* emerge in many parts of the world.

The most common mutations causing macrolide resistance are found in domain V in the 23S rRNA gene in positions 2058 and 2059 (*Escherichia coli* numbering). A2058G and A2059G are the most common mutations reported, followed by A2058T and A2058C. Similar mutations can be found in *Mycoplasma pneumonia* (*M. pneumoniae*).

4. Description

This assay detects *M. genitalium* DNA and in addition allows to predict antibiotics (macrolide) resistance based on 23S RNA gene mutations. A 77 bp long fragment targeting the *M. genitalium* gap gene is amplified with specific primers and detected with a FAM labeled hydrolysis probe.

Simultaneously a 115 bp long fragment from the *Mycoplasma ssp.* 23S RNA gene is amplified. This allows the detection of genetic variations associated to macrolide resistance using melting curve analysis with a probe matching the macrolide-sensitive wild type. The 23S RNA gene mutation test allows the resistance typing of various *Mycoplasma* species including *M. genitalium* and *M. pneumoniae*.

5. Specification

This assay detects 10 genome equivalent copies or less *M. genitalium* gap gene and 100 genome equivalent copies or less *Mycoplasma ssp.* 23S RNA gene per reaction (plasmid DNA dilution).

6. Sample Material and Extraction

Typical sample types for *M. genitalium* are genital 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
- 1: Cycling: PCR-amplification
- 1: Cooling: cooling the instrument

Instrument Settings

Set Quant Factor 10, Max Integration Time 1 sec

Detection Format

Hydrolysis Probe or SimpleProbe

LightCycler® 480 Instrument:

483-533

LightCycler® 480 II Instrument:

465-510

cobas z 480 Analyzer:

465-510

Table 1: Cycling condition programming including Melting.

Program Step:	Denaturation	Cycling			Melting			Cooling
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	45			1			1
Target °C	95	95	60	72	95	40	85	40
Hold hh:mm:ss	00:05:00	00:00:05	00:00:15	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
Ramp Rate °C/s 96	4.4	4.4	2.2	4.4	4.4	1.5		1.5
Ramp Rate °C/s 384	4.6	4.6	2.4	4.6	-	-		2.0
Acquisition Mode	None	None	Single	None	-	-	Continuous	None
Acquisitions per °C							3*	

* Melting slope shall be 0.19 to 0.29°C per second. If reading more channels reduce the number of acquisitions/sec.

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 where 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 **yellow** 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 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	Roche 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 µl (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)

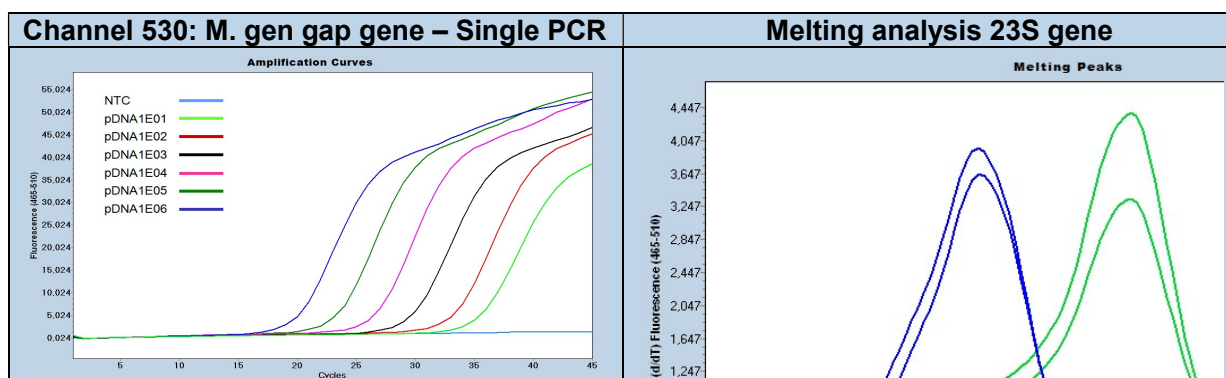


Figure 1: Dilution row 1E6 to 1E1 copies / reaction

Blue curves: SNP associated with Macrolide resistance
Green curves: SNP associated with Macrolide sensitivity

9. Reading the Results

Perform data analysis as described in the operator's manual. For multiplex assays select the color compensation for amplification and melting analysis. We recommend using the Second Derivative Maximum method (Automated (F'' max) for amplification and Tm Calling for melting analysis. View results in the FAM channel. The NTC must show no signal.

Table 3: Result analysis for individual channels.

Channel 530 (sample)	Channel 660 Control Reaction	Channel 530 NTC	Result
No amplification	Detectable	Negative	Not detectable
Amplification Cp < 39 ⁺	Not relevant	Negative	<i>M.genitalium</i> Positive
Low Melting Tm ~56 °C	Not relevant	Negative	Macrolide-resistant
High Melting Tm ~64 °C	Not relevant	Negative	Putative sensitive
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

Development of a quantitative real-time PCR assay for detection of Mycoplasma genitalium. Svenstrup et al., 2005
 A Novel SimpleProbe PCR Assay for Detection of Mutations in the 23S rRNA Gene Associated with Macrolide Resistance in Mycoplasma genitalium in Clinical Samples. Gossé, et al., 2016

11. Multiplex PCR Compatibility (STI Panel)

The *M. genitalium* + *Macrolide* assay can be combined with other assays up to 6plex reactions including an internal control or a spiked extraction control (for example PhHV) as depicted below:


Table 5: Options for combining M. gen+Macro in a multiplex PCR.

STI Multiplex PCR and Instrument Compatibility						480 II	z 480	LC96
Color Compensation 40-0320 is mandatory for Multiplex PCR								
500	530	580	610	640	660			
	M. gen+Macro					X	X	X
<i>C. trachomatis</i>	M. gen+Macro *	<i>T. vaginalis</i>	NG opaD	NG gyrA		X		
<i>C. trachomatis</i>	M. gen+Macro *	<i>T. vaginalis</i>	<i>M. hominis</i>	<i>Ureaplasma</i>	PhHV	X		
<i>C. trachomatis</i>	M. gen+Macro *	<i>T. vaginalis</i>	NG opaD	<i>T. pallidum</i>		X		
<i>T. pallidum</i>	M. gen+Macro *	<i>T. vaginalis</i>	NG opaD	<i>H. ducreyi</i>		X		

* Melting curve analysis run work in the multiplex PCR with all channels only if the Color Compensation is applied!

12. Version History

V210707	Melting probe shortened	2021-07-07
V220707	Figure for new probe and Tm ranges	2022-07-07
V230627	Editorial changes	2023-06-27
V231004	Roche SAP number included, chapters renumbered, editorial changes	2023-10-04

Certificate of Analysis (CoA)							
Lot n° 5112XXXX Expiry : YYYY-MM-DD							
Dilution	PC wt		1E4	PC	1E2	1E1	passed
Tm		Cp					
Measured:		Measured:					✓
Signal level		Signal level					
Measured		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 (Δ Cp). The values of the respective melting temperatures (Tm) may vary ± 2.5 °C between experiments and different 480 Instruments, while the distance between wt and mut should be relative constant (Δ Tm).							
DOM (manufactured): YYYY-MM-DD			QC Acceptance: 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) :							
Name 1				Name 2			

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