



MOLBIOL

**LightMix<sup>®</sup> in-vitro diagnostics kit**  
***Mycoplasma genitalium / hominis***  
***and Ureaplasma***

Cat.-No.: 40-0460-32

Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*,  
*Ureaplasma* genomic DNA

for use with the

Roche Diagnostics LightCycler<sup>®</sup> Instruments

Reagents for 96 reactions

**Upon arrival:**

**Store Premixed PCR reagents and Controls**  
**protected from light at room temperature (do not freeze)**

**Store FastStart DNA Master HybProbe reagents frozen (-20°C)**  
**(if included)**



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# 1. Product Information

## 1.1. Intended Use

This device is an *in-vitro* nucleic acid amplification test for the detection of genomic DNA from *Mycoplasma genitalium*, *M. hominis* and *Ureaplasma* from nucleic acid extracts obtained from human urogenital swabs.

The present diagnostic device must be used by qualified personnel only.

Note: The performance of the assay can be guaranteed only when used with LightCycler® Instruments (see 1.2.2 for details).

## 1.2. Specifications

### 1.2.1. Clinical Samples

Running the test requires 5 µl of purified DNA in aqueous solution.

The test is performed on DNA extracted from human urogenital swabs.

Before performing samples extraction read **5. Sample Preparation**.

The quantity of biological sample obtained with swabs is highly dependent on the collection procedure therefore absolute quantification is impossible.

### 1.2.2. Instruments, Software and Productivity

One kit contains reagents for 96 reactions performed in a 20 µl volume.

Each run requires two positive controls and one negative control.

The table below summarizes some features of the kit :

LightCycler® Instrument	Software Version (or higher)	Run Time (approx.)	Max Samples per run <sup>(1)</sup>	Maximum Productivity of the kit <sup>(1)</sup>	Minimum Productivity of the kit <sup>(1,2)</sup>
LC 2.0	4.05	70 min	29 + 3 ctrl.	87	24
LC480 II <sup>(4)</sup> (96 wells)	1.5	100 min	93 + 3 ctrl.	93	24
LC480 II <sup>(4)</sup> (384 wells)	1.5	100 min	381 <sup>(3)</sup> + 3 ctrl.	93	24
z 480 (open channel)	1.5	100 min	93 + 3 ctrl.	93	24

1 Each run includes 3 control reactions: two positive controls and one No-Target Controls (NTC).

2 Calculated considering a single clinical sample analyzed in each run.

3 It requires using four kits.

4 LightCycler® 480 "first version" cannot be used with this kit.

Table 1.

### 1.2.3. LightCycler® Instruments Channel (Filter) Settings

Instrument	Dye / channel name			
	530	LC610	LC640	LC690
LightCycler® 2.0	530	610	640	705
LightCycler® 480 II	465-510	498-610	498-640	498-660
cobas z 480 Analyzer	465-510	498-610	498-645	498-700

Table 2.

### 1.3. Contents

#### Lyophilized premixed PCR reagents

 **Store at room temperature in the dark**

Cap color	Label	Description content	Reaction Tube status	Total
3 x	Green	PSR Parameter Specific Reagents (PSR): Primers and hybridization probes specific for: <b><i>M. genitalium, M. hominis, and Ureaplasma</i></b> <0,01pg unlabeled oligonucleotides; <0,01pg Fluorescein-labeled oligonucleotides <0,01pg LightCycler Red 640 labeled oligonucleotides (M. g.) <0,01pg LightCycler Red 640 labeled oligonucleotides (M. h.) <0,01pg LightCycler Red 610 labeled oligonucleotides (U)	32 reactions Lyophilized blue-green pellet	96 rxs
3 x	White	CTR Control Reaction Reagents (CTR): Primers and hybridization probes specific for: <b><i>Extraction Control</i></b> <0,01pg unlabeled oligonucleotides <0,01pg LightCycler Red 690 labeled oligonucleotides; <0,01pg Fluorescein-labeled oligonucleotides.	32 reactions Lyophilized blue-green pellet	96 rxs

#### Control DNA

 **Store at room temperature**

Cap color	Label	Description content	Reaction Tube status	Total
1 x	Blue	Pos Ctr Mg-U <b>Positive Control for <i>M. genitalium</i> and <i>Ureaplasma</i></b> <0,01pg plasmid target (synthetic) [1E4 copies / reaction each]	32 reactions Lyophilized blue pellet	32 rxs
1 x	Yellow	Pos Ctr Mh <b>Positive Control for <i>M. hominis</i></b> <0,01pg plasmid target (synthetic) [1E3 copies / reaction]	32 reactions Lyophilized blue pellet	32 rxs
1 x	White	nECT <b>Extraction Control Target</b> <0,01pg plasmid target (synthetic) [4.8E6 copies: total amount]	100 reactions lyophilized blue pellet	96 rxs
1 x	Black	NTC <b>No Template Control</b> Stabilizer (DNA)	blue pellet	---

#### Polymerase Mix: LightCycler® FastStart DNA Master HybProbe

 **Store at -20°C upon arrival**

FastStart DNA Master HybProbe is included in kits supplied directly by TIB MOLBIOL for customers in Central Europe only <sup>(1)</sup>.  
The FastStart DNA Master HybProbe is not included in any kits supplied through Roche Diagnostics or its local distributor.

Cap color	Label	Description Content	Reaction Tube storage	Total
3 x	Red	1a LightCycler® FastStart Enzyme	32 reactions frozen	96 rxs
3 x	White	1b LightCycler® FastStart Reaction Mix Hyb-Probe	32 reactions frozen	96 rxs
3 x <sup>(2)</sup>	Colorless	Water H <sub>2</sub> O PCR grade	frozen	96 rxs
3 x <sup>(2)</sup>	Blue	MgCl <sub>2</sub> MgCl <sub>2</sub> , 25 mM	32 reactions frozen	96 rxs

1) FastStart enzyme is shipped by TIB MOLBIOL at ambient temperature.

2) FastStart enzyme supplied through Roche Diagnostics contains only 2 tubes of H<sub>2</sub>O and 1 tube of MgCl<sub>2</sub>, nevertheless the quantity provided is sufficient for the use described for this kit.

Table 3.

## 1.4. Storage and Stability

Note the **different storage conditions** for reagents and polymerase mix!

### Storage Conditions

#### Reagents and Controls

Store the lyophilized reagents (premixed PCR reagents and Control DNA) protected from light and at room temperature (18°C / 25°C).

Do not freeze these dried reagents. Expiration date is printed on the kit label.

#### Polymerase mix

Store the LightCycler® FastStart DNA Master HybProbe at -15°C to -25°C.

See expiration date on the polymerase tube label.

#### Shipping

Products are shipped at ambient temperature (no cooling). Transport stability of reagents and Roche FastStart enzyme components have been tested under common European shipping conditions (30°C and less). The dried reagents (PSR) have been tested to tolerate temporary incubation like two days at 60°C as to be expected to happen in Arabian countries.

## 2. Additional Devices and Reagents

### 2.1. Required

LightMix<sup>®</sup> Kit –Color Compensation HybProbes

**TIB Molbiol**  
Cat.-No. 40-0318-00

#### LightCycler<sup>®</sup> 2.0 Instrument

LightCycler<sup>®</sup> 2.0 Instrument  
LightCycler<sup>®</sup> Software Version 4.05 or  
LightCycler<sup>®</sup> Software Version 4.10 or higher  
LightCycler<sup>®</sup> Capillaries (20 µl)  
or

**Roche Diagnostics**  
Cat.-No. 12 011 468 001  
Discontinued  
Cat.-No. 04 779 584 001  
Cat.-No. 11 909 339 001

#### LightCycler<sup>®</sup> 480 II Instruments

LightCycler<sup>®</sup> 480 II Instrument  
cobas 4800 System (cobas z 480 Analyzer)  
LightCycler<sup>®</sup> Software Version 1.5 or higher  
LightCycler<sup>®</sup> 480 Multiwell Plate 96 white or  
LightCycler<sup>®</sup> 480 Multiwell Plate 384 white

**Roche Diagnostics**  
Cat.-No. 05 015 278 001  
Cat.-No. 05 200 881 001  
Cat.-No. 04 994 884 001  
Cat.-No. 04 729 692 001  
Cat.-No. 04 729 749 001

#### General materials

Phosphate Buffer Saline (PBS)  
Nuclease-free PCR grade water

any supplier  
any supplier

### 2.2. Optional

Extraction Control Target (nECT)

**TIB Molbiol**  
30-0259-96

LC Carousel Centrifuge 2.0 (230 Volt)  
Capping Tool

**Roche Diagnostics**  
Cat.-No. 03 709 582 001  
Cat.-No. 03 357 317 001

### 2.3. Sample Preparation

#### Manual Sample Preparation:

High Pure PCR Template Preparation Kit <sup>(#)</sup>  
or High Pure Viral Nucleic Acid Kit <sup>(#)</sup>  
Ethanol p.a.  
Isopropanol p.a.

**Roche Diagnostics**  
Cat.-No. 11 796 828 001  
Cat.-No. 11 858 874 001  
any supplier  
any supplier

#### Automatic Sample Preparation:

MagNA Pure 96 Instrument  
MagNA Pure 96 DNA and Viral NA Small Volume Kit  
MagNA Pure 96 IVD Instrument  
MagNA Pure 96 DNA and Viral NA Small Volume Kit  
MagNA Pure Instrument <sup>(#)</sup>  
MagNA Pure LC DNA Isolation Kit I  
MagNA Pure 2.0 Instrument <sup>(#)</sup>  
MagNA Pure LC DNA Isolation Kit I  
MagNA Pure Compact Instrument <sup>(§)</sup>  
MagNA Pure Compact Nucleic Acid Isolation Kit I  
X480 Instrument <sup>(#)</sup>  
cobas<sup>®</sup> 4800 System Sample Preparation Kit and  
cobas<sup>®</sup> 4800 System Wash Buffer Kit

**Roche Diagnostics**  
Cat.-No. 05 195 322 001  
Cat.-No. 05 467 497 001  
Cat.-No. 06 541 089 001  
Cat.-No. 06 543 588 001  
Discontinued  
Cat.-No. 03 003 990 001  
Cat.-No. 05 197 686 001  
Cat.-No. 03 003 990 001  
Cat.-No. 03 731 146 001  
Cat.-No. 03 730 964 001  
Cat.-No. 05 200 890 001  
Cat.-No. 05 235 782 190  
Cat.-No. 05 235 863 190



<sup>(#)</sup> Additional Extraction Control Target tube is required.

<sup>(§)</sup> The use of MagNA Pure Compact Instrument allows to perform only the **Internal Control** procedure (see 3.3.2).



## 3. Background Information

### 3.1. Medical Background

*Mycoplasma* and *Ureaplasma* are difficult to grow; NAT based tests such as PCR are the primary detection methods.

*Mycoplasma* are the smallest bacteria and have the smallest genome known. Since they lack a cell wall they are unassailable for the common beta-lactam antibiotics targeting cell wall synthesis. Infection with *Mycoplasma* is suspected to cause pelvic inflammatory disease and even infertility.

*M. genitalium* (MG) can be found on the ciliated epithelial cells of the genital but also respiratory tracts, causing discharge, burning while urinating, urethritis in men, and vaginal itching, and vaginosis in women. MG infections are relative rare.

*M. hominis* (MH) is part of the commensal gastrointestinal microbiota; infections of the urogenital tract in adults are very common and mostly symptomless.

*Ureaplasma* (*U. urealyticum* and *U. parvum*) are bacteria from the same family *Mycoplasmataceae*. They are part of the normal genital flora and transmitted horizontally, but also during birth. *Ureaplasma* has been associated with urethritis, infertility, premature birth, chorioamnionitis and stillbirth.

Infection of premature infants and newborns with *Mycoplasma* or *Ureaplasma* can cause pneumonia, sepsis, meningitis and branchopulmonary dysplasia.

Preferred specimens are genital swabs; urine has been reported to be insufficient.

### 3.2. Methodology and Assay Principle

This device amplifies and detects fragments from the genomes of *M. hominis*, *M. genitalium* and *Ureaplasma* from a nucleic acid extract obtained from swabs.

After every PCR cycle the probes bind during the annealing step to the PCR generated target and bring the fluorophores in close proximity. The donor fluorophore fluorescein is excited by light, part of the energy is transferred to the acceptor dye and detected. The fluorescence intensities are plotted against the cycle number, giving a picture of the amplification and calculating a crossing point (Cp) which is related to the log of the concentration of target in the sample.

Hybridization Probes can be used to identify the PCR product in a melting curve analysis in order to verify that the right product has been formed. The melting point (T<sub>m</sub>) is dependent on length and G+C content, but also upon the degree of homology to the sensor probe. In this device the T<sub>m</sub> is used for discrimination; slight temperature shifts are acceptable as the shape of curve depends also on the amount of product.

***M. genitalium*:** A 224 bp long fragment of the glyceraldehyde-3-phosphate dehydrogenase (gap) gene is amplified with specific primers and detected with specific probes labeled with the dye LightCycler<sup>®</sup> Red 640; the expected melting temperature (T<sub>m</sub>) in the melting curve analysis is 67-69°C.

***M. hominis***: A 129 bp long fragment of the gap gene is amplified with specific primers and detected with probes labeled with LightCycler® Red 640; the expected melting temperature is 59-61°C or about 56°C (common sequence variant).

***Ureaplasma***: A 187 bp long fragment from the 16S RNA gene is amplified with specific primers and detected with probes labeled with LightCycler® Red 610; the expected melting temperature is in the range of 64-67°C.

Any significant decrease in the melting temperature is an indication of a sequence variation, explaining an eventual lower signal level in the quantification plot and may give cause for a subsequent analysis of the sample or PCR product by DNA sequencing (feel free to contact us at: [service@tib-molbiol.de](mailto:service@tib-molbiol.de) for assistance).

The supplied control DNAs allow comparison with unknown patient samples.

### 3.3. Control Reaction

An additional PCR product of 349 bp is amplified from the Extraction Control Target (nECT) and detected with LightCycler® Red 690. The control reaction does not interfere with the analytical reaction; it is designed to be more sensitive to the presence of inhibitors and it will fail to amplify in presence of high-positive samples. The control reaction must be visible in negative and low-concentrated samples while it will fail in the presence of higher amounts of target DNA (1,000 copies/rxn or higher).

The Extraction Control Target can be utilized with two different procedures: either as Spiked Extraction Control (3.3.1) or as Internal Control (3.3.2).

	Pathogen			
	<i>Ureaplasma</i>	<i>M. hominis</i>	<i>M. genitalium</i>	Control
Channel	LC610	LC640	LC640	LC690
PCR Fragment	187 bp	129 bp	224 bp	349 bp
Melting curve Tm	64-67°C	59-61 /≈ 56°C	67-69°C	not relevant

Table 4.

#### 3.3.1. Spiked Extraction Control (sEC)

The spiked Extraction Control (sEC) procedure monitors the extraction and evaluates the presence of amplification inhibitors (recommended procedure).



The recovery rate of the purified plasmid present in the nECT is dependent of the extraciton procedure used; the amount of nECT must be adjusted accordingly. See detailed instruction in 5.1.

#### 3.3.2. Internal Control (IC)

The Internal Control (IC) procedure evaluates the presence of amplification inhibitors only.



This procedure is mandatory if extraction is performed with MagNA Pure Compact Instrument.



### 3.4. Performance Characteristics

#### Analytical Specificity

The specificity to the target gene and the suitability of the PCR amplification employed in the present test has been evaluated by direct DNA sequencing of the amplicon.

#### Analytical Sensitivity

Analytical sensitivity for cloned standards is 10 copies per PCR corresponding to 500 copies / mL clinical sample.

#### 3.4.1. Diagnostic Specificity, Sensitivity and Predictive Values

##### 3.4.1.1. *M. hominis*

Cell culture was chosen as the reference detection method for *M. hominis* (gold-standard). PCR is as expected more sensitive than culture; the numerous PCR- positive and cell culture negative samples were therefore accounted as false-positives. All discordant samples were analyzed for correction with two other independent PCR systems routinely used. To improve sensitivity, those PCRs were performed on a single genetic target and, to avoid any doubt, developed on different segments of the pathogen genome. Only samples positive to both PCR analyses were considered true positive and added to the Culture-positive samples.

		Culture + 2 independent PCRs				
			Neg	Pos	Prevalence	21.6%
TIB Molbiol	Mh	236	185	51	Sensitivity	100.0%
	Neg	184	184	0	Specificity	99.5%
	Pos	52	1	51	PPV	98.1%
					NPV	100.0%

##### 3.4.1.2. *Ureaplasma*

Cell culture is the reference gold-standard method also for *Ureaplasma*. For a correct identification of false positive samples, as above, all discordant samples were amplified also with other two independent PCR systems. Only samples positive to both PCR analyses were considered true positive and added to the Culture positive samples.

		Culture + 2 independent PCR				
			Neg	Pos	Prevalence	49.6%
TIB Molbiol	Uu/p	236	119	117	Sensitivity	98.3%
	Neg	118	116	2	Specificity	97.5%
	Pos	118	3	115	PPV	97.5%
					NPV	98.3%

##### 3.4.1.3. *M. genitalium*

*M. genitalium* does not grow in cell culture, therefore 4 different PCR systems were utilized to validate the samples with the same criteria described above.

		3 independent PCR				
			Neg	Pos	Prevalence	3.0%
TIB Molbiol	Mg	236	229	7	Sensitivity	85.7%
	Neg	229	228	<sup>(2)</sup> 1	Specificity	99.6%
	Pos	7	<sup>(1)</sup> 1	6	PPV	85.7%
					NPV	99.6%

(1) This sample was low positive only with two of the three PCRs therefore considered false positive.

(2) The quantity of *M. genitalium* was determined (~1E0 copies/ rx) by single target PCR; the same sample was also infected with *M. hominis* (~5E3 copies) and *Ureaplasma* (~2E3 copies).

## 4. Precautions and Warnings

### Handling Requirements

The present product is an *in-vitro* diagnostic device and therefore must be used by qualified personnel only.

General precautions for the handling of generic laboratory materials are required.

The laboratory work-flow must conform to standard practices. Due to the risk of contamination, PCR preparation and PCR amplification must be performed in physically separated areas.

Do not mix reagents from different lots.

Do not use the reagents after the expiration date.

Use the manual version which is delivered with the kit (see kit label).

### General Laboratory Procedures

All materials of human origin and related waste must be considered potentially infectious. Thoroughly clean and treat all work surfaces with disinfectants approved by local authorities.

Do not eat, drink or smoke in the laboratory working area.

Do not pipet by mouth.

Wear disposable protective gloves, laboratory coats and adequate eye protection during the handling of samples and set components.

Avoid microbial or nuclease contamination of the reagents while pipetting the aliquots. The use of disposable sterile tips with filter is essential.

Thoroughly wash your hands after handling the samples and the sets components.

### Sample Preparation

Regarding proper handling and disposal refer to the safety instructions enclosed in the package insert of the product employed (see chapter 2.3).

### Amplification and Detection

Before using this product, please read the LightCycler<sup>®</sup> Operator's Manual .

Save a sample file to identify each position for correct sample identification.

Check LightCycler<sup>®</sup> Instrument settings and make sure that they match those reported in the following section "PCR protocol" specific for your Instrument.

Do not touch the capillary surface or plate cover without gloves.

Please refer to all the operative and safety instructions of the LightCycler<sup>®</sup> Instrument.

### Handling of Waste Materials

Dispose of the unused reagents and waste materials according to the current laws.

## 5. Sample Preparation

The quantity of biological sample obtained with swabs is highly dependent on the collection procedure therefore absolute quantification is not possible.

### 5.1. Preparation of the Extraction Control Target (nECT)

Spin the tube at 10,000 RPM for 1 minute, dissolve the nECT in a PBS volume that depends on the extraction procedure used, as described below; mix and spin down (PBS is not supplied with the kit).

Extraction Procedure	Cat. No.	PBS
Internal Control procedure	(see 3.3.2).	1200 µl
High Pure PCR Template Preparation Kit	11 796 828 001	600 µl
High Pure Viral Nucleic Acid Kit	11 858 874 001	600 µl
MagNA Pure 96 DNA and Viral NA Small Volume Kit	05 467 497 001	1200 µl
MagNA Pure 96 DNA and Viral NA Small Volume Kit	06 543 588 001	1200 µl
MagNA Pure LC DNA Isolation Kit I	03 003 990 001	600 µl
cobas <sup>®</sup> 4800 System Sample Preparation Kit (§)	05 235 863 190	600 µl
MagNA Pure Compact Nucleic Acid Isolation Kit I(#)	03 730 964 001	1200 µl

(§) See 5.3 for specific instructions.  
(#) Only the use of the **Internal Control procedure** is allowed (see 3.3.2).




Table 5.

#### 5.1.1. Use of nECT as spiked Extraction Control (recommended)

When running the spiked Extraction Control procedure (sEC), add 10 µl of **Extraction Control Target** to the sample to be extracted.

Remember to complete No Target Control (NTC) and Diluents for Control DNAs (DIL) by adding nECT too (see 7.1.3).

#### 5.1.2. Use of nECT as Internal Control

If running the Internal Control Procedure, use the Extraction Control Target **only** in the preparation of the Reaction mix (see 7.2).

## 5.2. Swabs

### 5.2.1. Swabs in transport medium

Use 200 µl transport medium.

Perform nucleic acid purification with the appropriate extraction kit as described in the respective extraction protocols and extract into 50 µl.

### 5.2.2. Swabs in agarose

If vaginal or urethral swab arrives to the testing laboratory in agarose, disperse the swab in 250µl of PBS, let the particles of agarose to sediment for 3-5 minutes (agarose is a known PCR inhibitor), consider supernatant as transport medium.

## 5.3. X480 instrument

Spike the deep well plate with 10µl of nECT; utilize 400 µl of transport medium for extraction and set the elution to 100 µl.

Store purified DNA refrigerated (4°C - 8°C),  
if stored over a longer period of time keep frozen at -20°C.



## 6. Programming

### 6.1. Color Compensation



Color Compensation is required for the use of the *LightMix® Kit Mycoplasma gen/hom* and *Ureaplasma*.

Analyzing data with 'Color Compensation' deactivated will generate invalid readouts of the results.

### 6.2. Capillary Based Instruments

For details see the LightCycler® Operator's Manual.

The protocol consists of four program steps (Tab.5):

1. **Denaturation** of sample and activation of the enzyme
2. **Cycling** PCR-amplification of the target DNA
3. **Melting** Identification of PCR amplified DNA sequence
4. **Cooling** of the Instrument

Step:	1	2			3			4
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Target [°C]	95	95	62	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
Ramp Rate [°C/s]*	20	20	20	20	20	20	0.2	20
Sec Target [°C]	0	0	55	0	0	0	0	0
Step Size [°C]	0	0	0.5	0	0	0	0	0
Step Delay [cycles]	0	0	1	0	0	0	0	0
Acquisition Mode	None	None	Single	None	None	None	Cont.	None

**Note:**

While programming maintain default software values: max. samples = 32 and capillary size = 20 µl. Set seek temperature = 40°C and channel = 640.

Store the program and the default values as '**RUN Template**' which can be loaded to start every run.

Just before starting the run, modify max. samples (default = 32) to the number of samples plus controls included in the run to prevent stopping of the instrument due to missing capillaries.

For instructions see the Operator's Manual

Table 6. Programming of capillary based Instruments.

### 6.3. Multiwell Based Instruments

LightCycler® 480 II and cobas z 480 Analyzer

For details see the LightCycler® Operator's Manual.

#### Detection Format: Multicolor

Please refer to the manual of:

LightMix® Kit Color Compensation HybProbes Cat. No. 40-0318-00

Reaction Volume: 20 µl



#### Programming:

The protocol consists of four program steps (Tab.6):

1. **Denaturation** of sample and activation of the enzyme
2. **Cycling** PCR-amplification of the target DNA
3. **Melting** Identification of PCR amplified DNA sequence
4. **Cooling** of the Instrument

Step:	1	2			3			4
Parameter								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	50			1			1
Target [°C]	95	95	62	72	95	40	85	40
Acquisition Mode	None	None	Single	None	None	None	Cont.	None
Hold [hh:mm:ss]	00:10:00	00:00:05	00:00:05	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	0.14	1.5
Ramp Rate [C° s] 384	4.6	4.6	2.4	4.6	4.6	2.0	0.14	1.5
Acquisitions [per °C]	-	-	-	-	-	-	1	-
Sec Target [°C]	0	0	55	0	-	-	-	-
Step Size [°C]	0	0	0.5	0	-	-	-	-
Step Delay [cycles]	0	0	1	0	-	-	-	-

#### Note:

Store the program and the default values as 'RUN Template' which can be loaded to start every run. Ensure to program only 1 acquisition per second instead the default value 3; more acquisitions reduce the slope of the melting curve, increase experiment time and cause kit malfunction.


Table 7. Programming of multiwell based instrument (96 well and 384 well formats)

## 7. Experimental Protocol

Program the Instrument before preparing the solutions (see 6. Programming and read the LightCycler® Operator's Manual for details).

### 7.1. Reagents Preparation

#### 7.1.1. LightCycler® FastStart DNA HybProbe Master

1	Keep LightCycler® FastStart Enzyme <b>1a</b> cold.	
2	Thaw the LightCycler® FastStart Reaction Mix <b>1b</b> by warming up the tube at 30°- 35°C for 3 - 5 minutes.	
3	Quickly spin tubes to collect drops.	
4	The solution must be free of particles.	
5	Add 60 µl of <b>1b</b> to the vial <b>1a</b> .	
6	Mix the solution carefully with a pipette. Do not vortex! Avoid the production of bubbles.	
7	Spin the tubes to collect drops.	
8	Use reagent to prepare the Reaction Mix (6.3).	
9	Store left over reagent at 4°C.	

#### 7.1.2. Preparation of lyophilized PSR and CTR reagents

▶	Each reagent tube is sufficient for 32 reactions.
1	Spin the premixed tube at 10,000 RPM for 1 minute.
2	Check that the blue pellet is located at the bottom.
3	To each tube add <b>66 µl</b> of PCR-grade <b>Water</b> .
4	Incubate for 20 sec at room temperature.
5	Vortex for 10 sec.
6	Spin the tubes to collect drops.

▶ Use 2 µl of reagent for a 20 µl PCR reaction.

#### 7.1.3. No Template Control (NTC) and Diluents (DIL)

Label a clean vial as **DIL**.

Fill the **NTC** vial and the **DIL** vial with PCR-Grade water and nECT as described:

Extraction Procedure	H <sub>2</sub> O	nECT
Internal Control procedure	<b>1000 µl</b>	---
High Pure PCR Template Preparation Kit	<b>975 µl</b>	<b>25 µl</b>
High Pure Viral Nucleic Acid Kit	<b>975 µl</b>	<b>25 µl</b>
MagNA Pure 96 DNA and Viral NA Small Volume Kit	<b>950 µl</b>	<b>50 µl</b>
MagNA Pure 96 DNA and Viral NA Small Volume Kit	<b>950 µl</b>	<b>50 µl</b>
MagNA Pure LC DNA Isolation Kit I	<b>975 µl</b>	<b>25 µl</b>
cobas® 4800 System Sample Preparation Kit	<b>975 µl</b>	<b>25 µl</b>
MagNA Pure Compact Nucleic Acid Isolation Kit I	<b>1000 µl</b>	---

Table 8.

**NTC** is sufficient for 32 reactions.

▶ Use **5 µl** of **NTC** for a 20 µl PCR reaction.

**Do not manipulate NTC in the same location where Positive Controls are used.**

**DIL** is sufficient for the preparation of all **standards / controls**:

it must be prepared fresh and disposed immediately after use.

**DIL**, is used in close proximity of Positive Controls, may become contaminate.

## 7.1.4. Preparation of Control DNAs

▶	Each <b>Control</b> reagent tube is sufficient for 32 reactions.
1	Spin the two tubes at 10,000 RPM for 1 minute.
2	Check that the blue pellet is located at the bottom of the tube.
3	Dissolve pellet by adding 160 µl <b>DIL</b> .
4	Incubate for 20 sec at room temperature.
5	Vortex for 10 sec. and spin the tubes to collect drops.

▶ Use **5 µl** of each **Control** for a 20 µl PCR reaction.

▶ All **Controls** must be used in each run.

**Please note:** Opening the vials may cause contaminations of the work-space (aerosol).

## 7.2. Preparation of the Reaction Mix

We recommend preparing 32 reactions to prevent storage of dissolved or activated reagents in varying volumes. See chapter 7.3 for storage and stability of dilute components. For the preparation of reaction mix for fewer samples, please go to step 7.2.2.

### 7.2.1. Preparation of 32 Reactions Mix



Use left column “**sEC**” for the spiked Extraction Control procedure (see 3.3.1 and 5.1).

Use right column “**IC**” for the Internal Control procedure (see 3.3.2).

Prepare the reaction mix in the PSR reagent tube:

		sEC	IC
<b>Components</b>		<b>32 reactions</b>	
Add:	To the <b>PSR</b> tube (green cap) already containing	66.0 µl	66.0 µl
	H <sub>2</sub> O, PCR-grade (colorless cap)	217.8 µl	201.3 µl
	Mg <sup>2+</sup> solution 25 mM (blue cap)	79.2 µl	79.2 µl
	<b>CTR</b> (white cap)	66.0 µl	66.0 µl
	LightCycler® FastStart DNA Master HybProbe (red cap), see 7.1.1	66.0 µl	66.0 µl
	<b>nECT</b> when used as Internal control (white cap), see 3.3.2	---	16.5 µl
	Substitute the “long neck cap” of the <b>PSR</b> tube with the red cap from FastStart		
<b>Total Volume</b>		<b>495.0 µl</b>	<b>495.0 µl</b>

Table 9. Volumes of components for preparing 32 reaction mixture



Gently pipette up and down the reaction mix.  
A high percentage of experimental failure is due to a non homogeneous reaction mix!



### 7.2.2. Preparation of a Single Reaction Mix



Use left column “**sEC**” for the spiked Extraction Control procedure (see 3.3.1 and 5.1).

Use right column “**IC**” for the Internal Control procedure (see 3.3.2).

Prepare the reaction mix by multiplying each volume by the number of biological samples to be analyzed plus four reactions (**NTC**, two **Controls**, one excess).

Prepare the reaction mix in a cooled vial:

	sEC	IC
<b>Components</b>	<b>Single reaction</b>	
H <sub>2</sub> O, PCR-grade (colorless cap)	6.6 µl	6.1 µl
Mg <sup>2+</sup> solution 25 mM (blue cap)	2.4 µl	2.4 µl
<b>PSR</b> (green cap), see 7.1.2	2.0 µl	2.0 µl
<b>CTR</b> (white cap), see 7.1.2	2.0 µl	2.0 µl
LightCycler® FastStart DNA Master HybProbe (red cap), see 6.2.1	2.0 µl	2.0 µl
<b>nECT</b> when used as Internal control (white cap), see 3.3.2	---	0.5 µl
<b>Volume of reaction mix</b>	<b>15.0 µl</b>	<b>15.0 µl</b>

Table 10. Volumes of components for preparing a single reaction mixture



Gently pipette up and down the reaction mix.  
A high percentage of experimental failure is due  
to a non homogeneous reaction mix!



## 7.3. Storage and Stability of Diluted Components

### Reaction Mix

The complete reaction mix containing lyophilized premixed PCR reagents, LightCycler® FastStart DNA Master HybProbe and MgCl<sub>2</sub> can be stored refrigerated (4°C - 8°C) for 30 days. Avoid prolonged exposure to light.

### Lyophilized premixed PCR reagents

Once diluted, store the reagents refrigerated at 4°C - 8°C for a maximum period of 30 days. Avoid prolonged exposure to light.

### LightCycler® FastStart DNA Master HybProbe

The combined FastStart mastermix (1a+1b) can be stored refrigerated (4°C - 8°C) for 30 days.

### Controls

The dissolved **Controls** are stable for 30 days when stored refrigerated (4°C - 8°C). When using them over a longer period of time, aliquot and store frozen at -20°C. Controls stored at -20°C are stable for 180 days.

### Note:

More than five (5) freezing and thawing cycles of controls may result in shift in the Cp value compared to the freshly dissolved vial; avoid the use of the control when the Cp value changes more than 1 cycle.



## 7.4. Loading of Controls and Samples

### 7.4.1. Samples attributes

Controls and samples attributes are summarized in the table below:

Number	Sample Name	Channel 2.0	Channel LC480II	Channel z 480	Quantification Sample Type
1	NTC	610	498-610	498-610	Negative
		640	498-640	498-645	Negative
		705	498-660	498-700	Positive for nECT
		610	498-610	498-610	Positive for <i>Ureaplasma</i>
		640	498-640	498-645	Positive for Mg
		705	498-660	498-700	Not relevant
3	Mh	610	498-610	498-610	Negative
		640	498-640	498-645	Positive for Mh
		705	498-660	498-700	Not relevant
4...	Sample	610	498-610	498-610	Unknown
		640	498-640	498-645	Unknown
		705	498-660	498-700	Unknown



The suggested loading order simplifies the programming of the 'Sample List' in the LightCycler® Instruments.

Table 11.

### 7.4.2. Capillary / Well Loading Procedure

Each run must include one Negative Control (**NTC**) to demonstrate the absence of contaminations with target DNA or PCR product and the **positive controls** to ensure the performance of the kit.

#### Capillary / Well Loading Procedure

1	Mix gently, spin down and check for the absence of air bubbles in the reaction mix vial.	
2	Dispense <b>15 µl</b> per capillary/well.	
3	<b>Remember to include the controls when setting up the run:</b> Add <b>5 µl</b> of <b>Negative Control (NTC)</b> (Black cap) Add <b>5 µl</b> of <b>Mg-U Control</b> (Blue cap) Add <b>5 µl</b> of <b>Mh Control</b> (Yellow cap)	
4	Add <b>5 µl</b> of <b>Sample</b> in the remaining capillaries / wells.	
5	Close the capillary/plate and centrifuge. Check that no air bubbles are present.	
6	Place the rotor/plate into the LightCycler® Instrument.	
7	Capillary-based users only: input number of samples.	
8	Start the run.	
9	Input experiment's name when instructed.	
10	Save sample data in the samples' window.	

### 7.4.3. Capillary Based Instruments

In "Samples data - Capillary View", input Sample Name as described in the second column of Table 9. Select "Analysis Type – Abs Quant". Select Channel 610, 640 and 705 only !

### 7.4.4. Plate Based Instruments

In the "Sample Editor" window, in "Step1: Select Workflow" section, select "Abs Quant". Input sample Name as described in the second column of Table 9.

## 8. Data Analysis and Interpretation

### 8.1. Limits and Interferences

The present test is specific for *Mycoplasma gen/hom* and *Ureaplasma*.

### 8.2. Calibration

Calibration has to be performed following the procedure described in 7.4, 8.3.1, 8.3.2 and 8.3.3.

### 8.3. Quality Control – Acceptance Criteria

In order to perform a reliable analysis, it is essential that Negative Control **NTC** and all **Controls** are included in each run.

The **CTR** reagent contains primers and probes for the amplification of a foreign DNA target (**nECT**) added to the PCR following the **spiked Extraction Control (sEC)** (see 3.3.1) or the **Internal Control (IC)** (see 3.3.2) procedures.

The control reaction must be visible in negative and low-concentrated samples while it will fail in the presence of higher amounts of target DNA (1,000 copies/PCR or higher).



Activate Color Compensation.

Analyzing data with 'Color Compensation' deactivated will generate invalid readouts of the results.

#### 8.3.1. Negative Control (NTC)

**NTC Negative Control** is mandatory – it mimics negative clinical samples.

Amplification analysis of NTC must provide a **negative result in channels LC610 and LC640**.

If amplification is visible in even one of these channels, a contamination or a pipetting error has occurred; the session is not valid and the whole procedure has to be repeated (amplification and detection).

If the problem sustains, change water and/or reagents and repeat.

**In channel LC690 NTC** must provide a **positive result**:

**nECT** amplification must be detected.

If the **nECT** amplification is absent, the session is not valid and the whole procedure must be repeated (amplification & detection).

### 8.3.2. Positive Control for *M. genitalium* and *Ureaplasma*

Inclusion of the **Pos. Control for *M. genitalium* and *Ureaplasma*** is mandatory: The positive control mimics positive samples.

#### 8.3.2.1. *Ureaplasma*

Amplification analysis of ***M. genitalium* and *Ureaplasma* Positive Control** must provide a **positive amplification** in channel LC610. The Cp value must conform with expected results (see 8.4). If amplification is absent see instruction in 8.3.4.

Melting-curve analysis of ***M. genitalium* and *Ureaplasma* Positive Control** must provide a melting curve with  $T_m = 65-67^\circ\text{C}$  in channel LC610.

#### 8.3.2.2. *M. genitalium*

Amplification analysis of ***M. genitalium* and *Ureaplasma* Positive Control** must provide a **positive amplification** in channel LC640. The Cp value must conform with expected results (see 8.4). If amplification is absent see instruction in 8.3.4

Melting-curve analysis of ***M. genitalium* and *Ureaplasma* Positive Control** must provide a melting curve with  $T_m = 67-69^\circ\text{C}$  in channel LC640.

### 8.3.3. Positive Control for *M. hominis*

Inclusion of the **Pos. Control for *M. hominis*** is mandatory: The positive control mimics positive samples.

Amplification analysis of ***M. hominis* Positive Control** must provide a **positive amplification** in channel 640. Cp must conform with expected results (see 8.4)

If amplification is absent see instruction in 8.3.4.

If any amplification signal for the ***M. hominis* Positive Control** is seen in the LC610 channel, the session is not valid and the whole procedure has to be repeated.

Melting-curve analysis of ***M. hominis* Positive Control** must provide a melting curve with a  $T_m$  of about  $59-61^\circ\text{C}$  in channel 640.

If a melting peak for *M. hominis* Positive Control is present in channel LC610, the session is not valid and the entire procedure has to be repeated (amplification and detection).

### 8.3.4. Failures in runs of Controls

Complete data from controls are essential to get accurate results with patient samples.

Runs without adequate results for the **controls** must be repeated.



Before repeating a run consider common errors; check in particular the amplification program, use of the correct master-mix and  $\text{MgCl}_2$  concentration; remember that also inadequate storage of reagents may cause a failure of the device.

### 8.3.5. Samples

#### Negative Clinical Samples

Amplification analysis provides **no amplification** signal in channels LC640 and LC610.

Amplification analysis in channel LC690 must provide the **nECT derived amplification** signal.

Melting-curve analysis provides **no melting curves** in channels LC640 and LC610.



**Clinical samples displaying neither target amplification nor nECT signal are invalid** and the whole procedure has to be repeated (sample preparation, amplification and detection).

#### Positive Clinical Samples for *Ureaplasma*

Amplification analysis provides a **positive amplification** in channel LC610.

Melting-curve analysis must yield a melting curve in channel LC610 with  $T_m = 66^\circ\text{C}$ .

#### Positive Clinical Samples for *M. genitalium*

Amplification analysis provides a **positive amplification** in channel LC640.

Melting-curve analysis must yield a melting curve in channel LC640 with  $T_m = 67\text{-}69^\circ\text{C}$ .

#### Positive Clinical Samples for *M. hominis*

Amplification analysis provides a **positive amplification** in channel LC640.

Melting-curve analysis must yield a melting curve in channel LC640 with  $T_m = 59\text{-}61$  or  $\sim 56^\circ\text{C}$ .

**IMPORTANT:** *M. hominis* positive samples having a melting curve with a  $T_m \sim 56^\circ\text{C}$  might not show an amplification signal; **always perform melting curve analysis!**



#### nECT in Clinical Samples

Amplification analysis provides a **positive amplification** in channel LC690 only in samples with a low copy number of *Mycoplasma/Ureaplasma*.

Melting-curve analysis is not relevant.



Multiple infection have been frequently observed.

### 8.3.6. Abnormal Melting Curves

Abnormal melting curves generated by variants are sometime reported; in this case another method must be used for verification of the amplified product.

Report deviations to [service@tib-molbiol.de](mailto:service@tib-molbiol.de).

Feel free to send deviant melting samples to TIB Molbiol Berlin laboratories to confirm the obtained results by DNA sequencing.

## 8.4. Reading the Results

Perform data analysis as described in the LightCycler® Operator's Manuals.



**Activate Color Compensation!**

Analyzing data with 'Color Compensation' deactivated will generate invalid readouts of the results.

### 8.4.1. Amplification Analysis: Capillary Based Instruments

View data for *Ureaplasma* in channel LC610, for *M. hominis* and *M. genitalium* in channel LC640 and for nECT in channel LC690, "Absolute Quantification" analysis mode.

	<p><b>LC610</b></p> <p><b>NTC</b> (light blue line) must show a flat line.</p> <p><b>U</b> Positive Control (blue line) must show an amplification signal. Typical Cp=24-26.</p> <p><b>Mh</b> Positive Control (orange line) must show a flat line.</p>
	<p><b>LC640</b></p> <p><b>NTC</b> (light blue line) must show a flat line.</p> <p><b>Mg</b> Positive Control (blue line) must show an amplification signal. Typical Cp=25-27.</p> <p><b>Mh</b> Positive Control (orange line) must show an amplification signal. Typical Cp=28-30.</p>
	<p><b>LC690</b></p> <p><b>NTC</b> (light blue line) must show an amplification signal.</p> <p><b>Mg-U</b> Positive Control (blue line) might show an amplification signal.</p> <p><b>Mh</b> Positive Control (orange line) might show an amplification signal. Typical Cp=28-32</p>

**Fig. 1: Amplification data for *Ureaplasma*.**

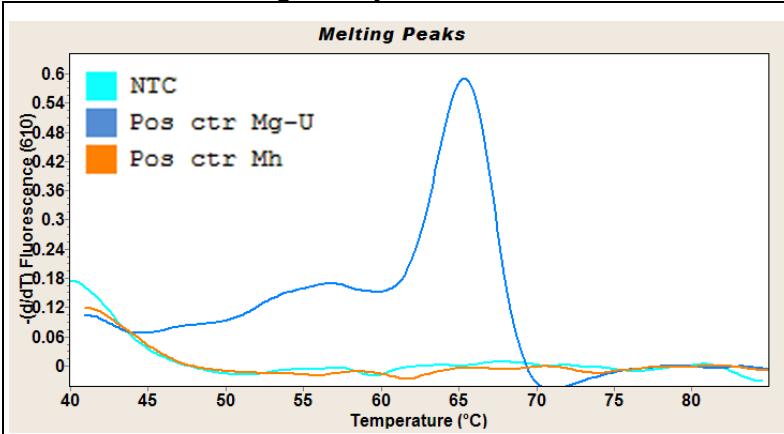
**Fig. 2: Amplification data for *M. genitalium* and *M. hominis*.**

**Fig. 3: Amplification data for sEC/IC**

**Note:** The values of the crossing point (Cp) may vary  $\pm 1.5$  cycles between different experiments. In case of variations see instructions: **8.3.4 Failures in runs of Controls.**

## 8.4.2. Melting Curve Analysis: Capillary Based Instruments

View data for *Ureaplasma* in channel LC610 and for *M. hominis* and *M. genitalium* in channel LC640, "Tm Calling" analysis mode.



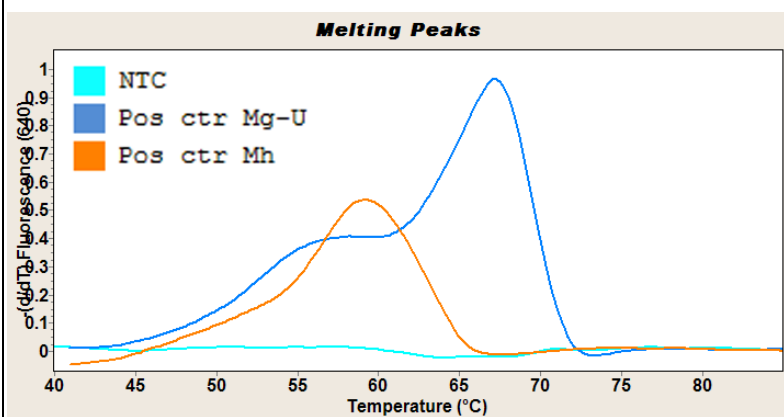
### LC610

**NTC** Negative Control (light blue line—position 1) no assay-specific melting peaks must be detected.

**U** Positive Control (blue line) shows a melting peak at 66°C.

**Mh** Positive Control (orange line) shows no melting peak.

**Fig. 4: Melting curve for *Ureaplasma*.**



### LC640

**NTC** Negative Control (light blue line—position 1) no assay-specific melting peaks must be detected.

**Mg** Positive Control (blue line) shows a melting peak at 68°C.

**Mh** Positive Control (orange line) shows a melting peak at 59-61°C.

***Mycoplasma hominis* samples frequently show a variant lower melting peak at 56°C (see 8.5 and fig. 10).**

**Fig. 5: Melting curve for *Mycoplasma genitalium* and *Mycoplasma hominis*.**

**Note:** The values of the melting temperatures (Tm) may vary  $\pm 2.5^\circ\text{C}$  between different experiments.

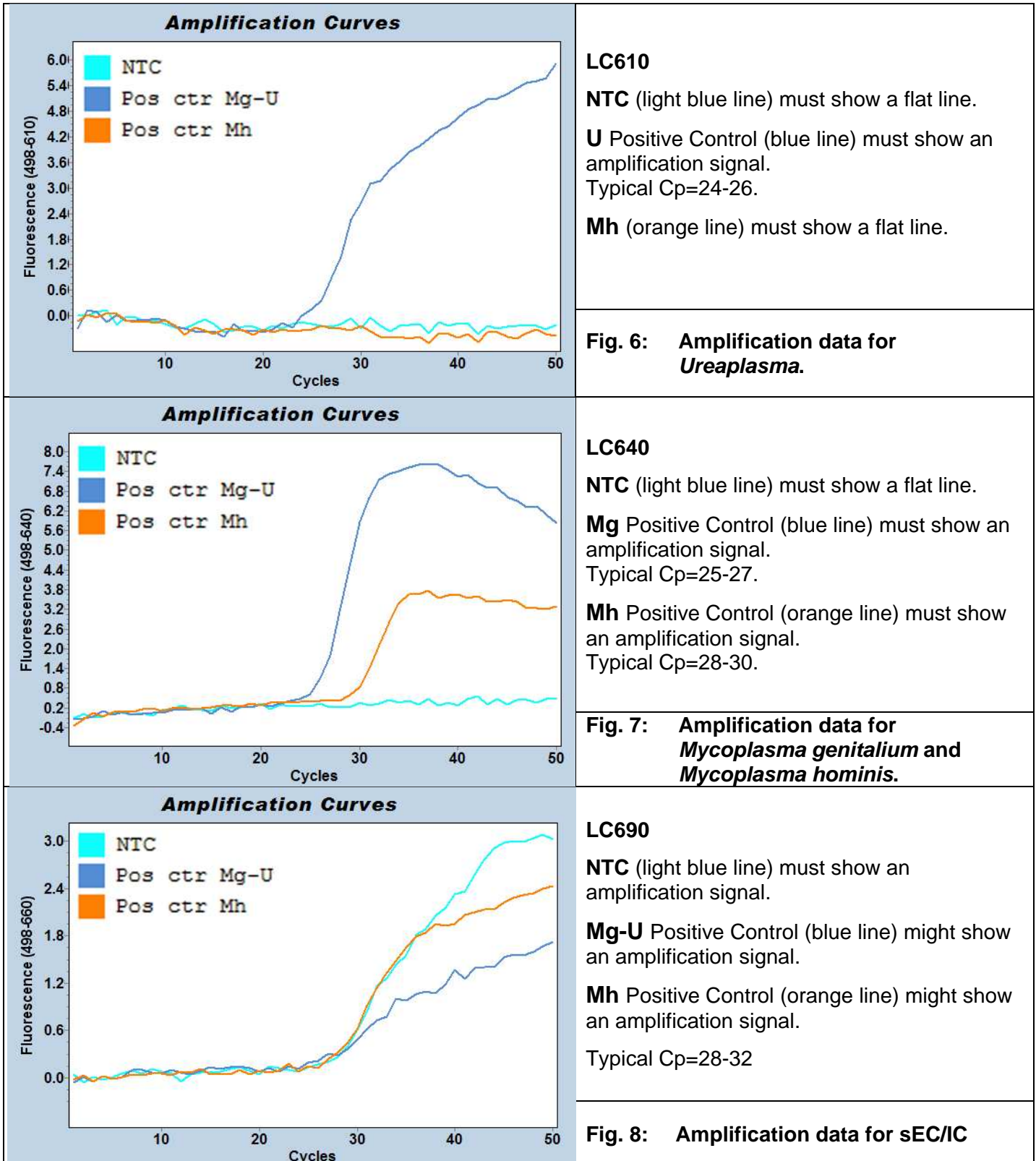


### nECT Melting curve in LC690 channel.

Melting curve for nECT in Positive control and positive samples is not relevant.

### 8.4.3. Amplification Analysis: Plate Based Instruments

View data for *Ureaplasma* in channel LC610, for *M. hominis* and *M. genitalium* in channel LC640 and for nECT in channel LC690 “Abs Quant/2nd Derivative Max ” analysis mode.



**Fig. 6: Amplification data for *Ureaplasma*.**

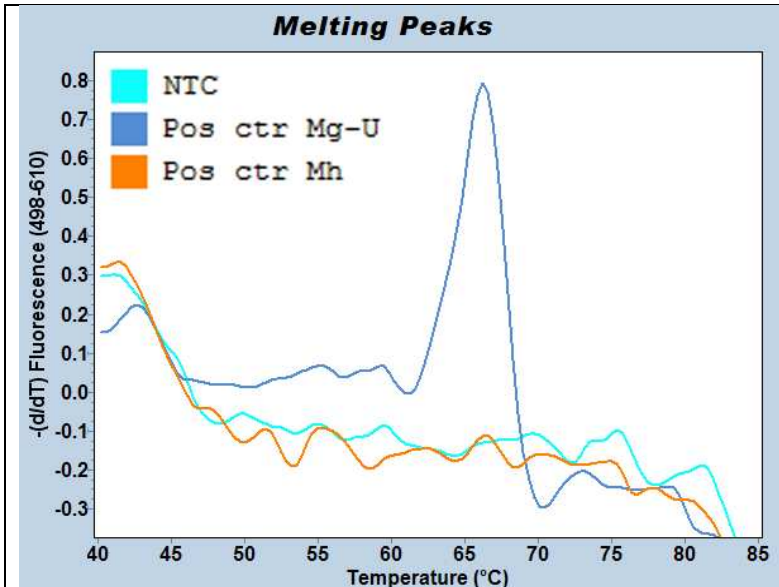
**Fig. 7: Amplification data for *Mycoplasma genitalium* and *Mycoplasma hominis*.**

**Fig. 8: Amplification data for sEC/IC**

**Note:** The values of the crossing point (Cp) may vary  $\pm 1.5$  cycles between different experiments. In case of variations see instructions: **8.3.2 Failures in runs of Controls.**

### 8.4.4. Melting Curve Analysis: Plate Based Instruments

View data for *Ureaplasma* in channel LC610 and for *M. hominis* and *M. genitalium* in channel LC640, "Tm Calling" analysis mode.



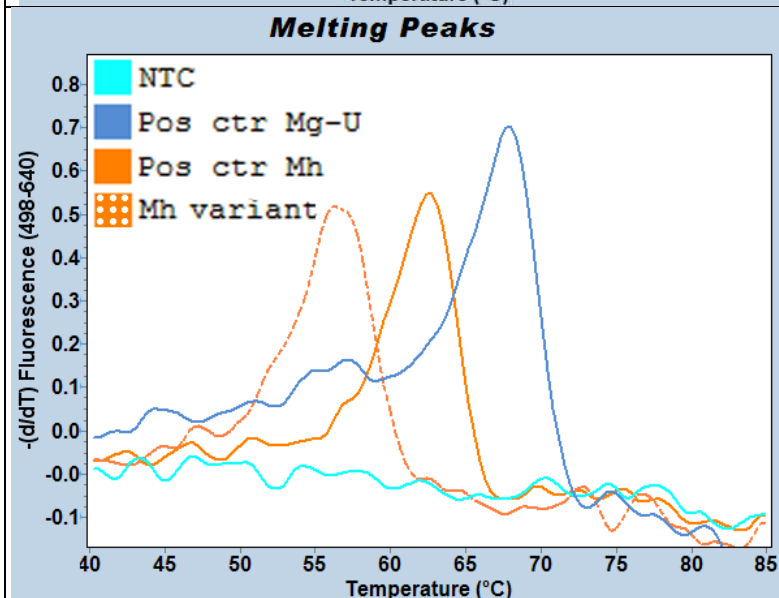
#### LC610

**NTC** Negative Control (light blue line—position 1) no assay-specific melting peaks must be detected.

**U** Positive Control (blue line) shows a melting peak at 66°C .

**Mh** Positive Control (orange line) shows no melting peak.

**Fig. 9: Melting curve for *Ureaplasma*.**



#### LC640

**NTC** Negative Control (light blue line—position 1) no assay-specific melting peaks must be detected.

**Mg** Positive Control (blue line) shows a melting peak at 68°C .

**Mh** Positive Control (orange line) shows a melting peak at 61°C .

***Mycoplasma hominis* samples frequently show a variant lower melting peak at 56°C.**

**Fig. 10: Melting curve for *Mycoplasma genitalium* and *Mycoplasma hominis*.**

**Note:** The values of the melting temperatures (Tm) may vary  $\pm 2.5^\circ\text{C}$  between different experiments.



#### nECT Melting curve in LC690 channel.

Melting curve for nECT in Positive control and positive samples is not relevant.



## 8.5. Interpretation Table

First check results of Controls: for a valid run all controls must be passed.

Controls			Results
Amplification	Amplification / Melting temperature	Amplification	
LC610	LC640	LC690	
<b>NTC</b>			
Negative	Negative	Amplification	Negative Control Passed
Negative	Negative	Negative	<b>nECT Failed</b> Check if sEC/IC was used !
Amplification	Amplification	not relevant	<b>Negative Control Failed <sup>(1)</sup></b> Contamination ! Repeat !
<b>Positive Controls Mg-U</b>			
Amplification	Amplification / Peak 67-69°C	not relevant	Mg-U Positive Control Passed
Negative	Negative	not relevant	<b>Mg-U Positive Control Failed</b>
<b>Positive Controls Mh</b>			
Negative	Amplification / Peak 59-61°C	not relevant	Mh Positive Control Passed
Negative	Negative	not relevant	<b>Mh Positive Control Failed</b>

Note: (1) If amplification is present in either channel (LC610 or LC640), Negative Control is failed.

Table 12.

Then read results for the clinical samples :

Clinical Samples			Results
Amplification	Amplification / Melting temperature	Amplification	
LC610	LC640	LC690	
Negative	Negative	Amplification	Negative for Mg, Mh and U. (not detectable)
Amplification	Negative	not relevant	<b>Positive for <i>Ureaplasma</i></b>
Negative	Amplification / Peak 67-69°C	not relevant	<b>Positive for <i>M. genitalium</i></b>
Negative	Amplification / Peak 59-61°C or 56° <sup>(2)</sup>	not relevant	<b>Positive for <i>M. hominis</i></b>
Negative	Negative	Negative	<b>Amplification Control Failed</b> Check if sEC/IC was used and repeat from extraction

Note: (2) *M. hominis* positive samples showing a melting peak at 56°C might not show an amplification signal (see 8.3.5)



Clinical samples with multiple infection will show corresponding amplification and melting data.

Table 13.

## 9. Troubleshooting

Instrument specific codes:	Capillary based instruments	Multiwell based instruments
Event	Possible Reason	Solution
No sample detected	No centrifugation	Centrifuge capillary
All negative PCRs	Incorrect selection of detection channel	Set correct channel before analyzing
	Incorrect amplification protocol	Check instrument program
Inconsistent baseline among various samples	Incorrect pipetting	Ensure homogeneity of mix quantity in each sample
	Non homogenous reaction mix	Pipette reaction mix up and down 10 x with a clean 200µl tip before distribution in reaction vessel
	Multiwell plate badly sealed	Ensure that multiwell plate is properly sealed
Baseline "Saw teeth like"	Non homogeneous mix	Centrifuge plate before run
	Incorrect positioning of capillary in the carousel	Firmly press capillary in the carousel
No signal in the Positive Control	Error while setting the instrument	Check the position settings of the Positive Control
	Incorrect PSR / MgCl <sub>2</sub> concentration	Repeat assay
	Positive Control or standard degradation	Use a new aliquot of Positive Control or standard
Positive signal in <b>NTC</b> Negative Control	Error while setting the instrument	Check the position settings of the Negative Control
	Dispensing error	Comply with the work sheet when dispensing samples, Negative Controls, Positive Controls and standards
	Dispensing error	Always change tips among samples
	Dispensing error	Avoid spilling the contents of the sample test tube
	Contamination of the PCR-grade water.	Use a new aliquot of PCR- grade water
	Contamination of the reaction mix	Use new aliquots of reagents to prepare the reaction mix
	Contamination of the extraction/preparation area for amplification reactions	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use
	Contamination of the extraction/preparation area for amplification reactions	Add LightCycler <sup>®</sup> Uracil-DNA Glycosylase (Cat.-No.03 539 806 001) to the reaction mix according to instructions
No signal in samples	Low DNA amount	Check DNA concentration
	Sample inhibition	Dilute sample and repeat PCR, or repeat extraction and PCR, or add Positive Control and repeat
Melting curve outside the expected temperature range	Possible altered target sequence	Verify by repeating, sequence the virus (if applicable). Report to: <a href="mailto:service@tib-molbiol.de">service@tib-molbiol.de</a> and/or send samples for further analysis to the laboratory in Berlin.

## 10. References

[1] Helle Friis Svenstrup, Jørgen Skov Jensen, Eva Bjo'rneelius, Peter Lidbrink, Svend Birkelund and Gunna Christiansen

**Development of a Quantitative Real-Time PCR Assay for Detection of Mycoplasma genitalium**

*JOURNAL OF CLINICAL MICROBIOLOGY*, July 2005, p. 3121–3128 Vol. 43, No. 7

[2] Robertson JA, Vekris A, Bebear C, Stemke GW.

**Polymerase chain reaction using 16S rRNA gene sequences distinguishes the two biovars of Ureaplasma urealyticum.**

J Clin Microbiol. 1993 Apr; 31(4):824-30

[3] Agata Baczynska, Helle F Svenstrup, Jens Fedder, Svend Birkelund and Gunna Christiansen

**Development of real-time PCR for detection of Mycoplasma hominis.**

BMC Microbiology 2004, 4:35

### Notice to Purchaser -- Patents and Trademarks

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### Classification / References

Reference	Classification
EDMA	15.30.04.49 (15.01.08.40) (15.01.40.90)
CPV	33694000-1
EAN	4260159332513
Roche SAP No.	07103913001

### FastStart Enzyme

FastStart DNA Master HybProbe is included in the package for TIB MOLBIOL customers in Central Europe only.

When the present kit is distributed through Roche Diagnostics or their local distributors, FastStart DNA Master HybProbe is supplied as a separate product:

Roche Diagnostics Cat.-No. 03 003 248 001 kit for 96 reactions

Roche Diagnostics Cat.-No. 12 239 272 001 kit for 480 reactions

## 11. Material Safety Data Sheet

According to OSHA 29CFR1910.1200, Commonwealth of Australia [NOHSC:1005, 1008 (1999)] and the European Union 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.

## 12. Version History

**Notes: in red mark events that require to change laboratory procedures**

**Notes in blue mark improvements and changes in composition**

Version	Event	Date
150430	Release Version	30-04-2015
150606	5.1 Detailed description for spiking procedure Corrected the quantity of nECT in table 7 and 8	11-09-2015
160116	X480 instrument allowed (Sections 2. and 5.3); modification of the preparation procedures of nECT (5.1), NCT and DIL (7.1.3); Internal Control procedure: changed nECT volume in the Master Mix preparation (7.2.1 and 7.2.2); Editorial changes.	16-01-2016

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