

# RevoDx Mycoplasma Genitalium

## qPCR Kit

## Product Manual

### Qualitative detection of Mycoplasma Genitalium DNA

For *in vitro* diagnostic use.

For professional use only

#### Product numbers:

IP202243-100 – 100 tests

IP202243-500 – 500 tests



### Product Components

	Component Name	100 Tests	500 Tests
1	M. Genitalium RM 1	1400 µl	5 x 1400 µl
2	M. Genitalium RM 2	100 µl	500 µl
3	M. Genitalium Positive Control	100 µl	200 µl
4	M. Genitalium Negative Control	100 µl	200 µl

### Transport, Storage and Stability

The kits may be shipped at +2°C to +8°C. All components of RevoDx Mycoplasma Genitalium qPCR Kit should be stored at -25°C to -15°C. Storage at higher temperatures should be avoided. If properly stored, all kit components are stable until the expiration date printed on the product label. M. Genitalium RM 1 and RM 2 vial should not be freeze-thawed more than 3 times as this may reduce the sensitivity. Otherwise, divide them into conveniently sized aliquots, and store at -25°C to -15°C.

### Intended Use

RevoDx Mycoplasma Genitalium qPCR Kit is a real-time PCR test intended for the qualitative detection of Mycoplasma Genitalium DNA.

Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. Negative results do not preclude the infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

RevoDx Mycoplasma Genitalium qPCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

### Product Use Restrictions

- RevoDx Mycoplasma Genitalium qPCR Kit is for *in vitro* diagnostic use only.
- Potential mutations in the target regions of the Mycoplasma Genitalium genome covered by the oligos in the kit may lead to false negative test results.
- PCR inhibitors in eluates may lead to false negative or invalid test results.
- Reliable results depend on proper specimen collection, transport, storage and handling methods.
- It is intended for professional use by properly trained personnel.
- Follow the instructions in product manual for optimum PCR results.
- Do not use a kit after its expiration date. Kit components from different lots should not be mixed.

### Product Description

Mycoplasma Genitalium Real-Time PCR assay is a fluorogenic probe-based PCR assay in which, situated between two PCR primers, there is an internal oligonucleotide probe with a fluorescent label attached at the 5'-end and a quenching molecule that suppresses the fluorescent reporter at the 3'-end. During DNA replication in the PCR process, the internal oligonucleotide hybridizes to the template and is digested by the 5'-3' endonuclease activity of the *Thermus aquaticus* (Taq) DNA polymerase as the PCR primer is extended. The internal oligonucleotide is digested only if DNA replication occurs, separating the fluorescent and quencher molecules. PCR products are detected within minutes by monitoring the increase in fluorescence that occurs exponentially with successive PCR amplification cycles. The parameter Ct (threshold cycle) is defined as the fractional cycle number at which the fluorescence passes the fixed threshold. Mycoplasma Genitalium real-time PCR assay utilizes an internal control, which controls for target isolation and amplification.

### General Description

Mycoplasma genitalium is the smallest known self-replicating bacterium. It lacks a cell wall and hence cannot be detected on Gram staining of a specimen. M. genitalium is predominantly found in the genitourinary tract of both sexes. It evades the adaptive immune system by establishing intracellular infection (possibly due to its tip-like structure), but also by antigenic and phase variation of its surface-expressed proteins. M. genitalium infection may persist for months or years in asymptomatic individuals. It is found in genital specimens of 15-25% of men with non-gonococcal urethritis and 10-13% of women with pelvic inflammatory disease. Although the pathological consequences of M. genitalium infection are thought mainly to be due to host response, M. genitalium has been shown to directly cause ciliary damage in human fallopian cell culture.

### Safety Information

- Clinical specimens should be treated as potentially infectious; they should be handled in Bio-safety Level 1 or Bio-safety Level 2 area, depending on the infective agents.
- All resulting waste should be considered potentially infectious. They should be handled and discarded according to local safety regulations.
- Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.
- Avoid producing spills or aerosol.
- Never pipette solutions by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands after handling samples and test reagents.
- All MSDS information is available upon request.
- When working, always wear a protective lab coat, disposable gloves and protective goggles.
- Before and after procedure, disinfect all work surfaces thoroughly with a freshly prepared solution of 10% bleach or antiviral agents.
- Make sure everything is DNase/RNase-free when handling this system.
- Handle all materials according to Good Laboratory Practices in order to prevent cross-contamination.
- Use only calibrated pipettes, always change pipette tips between liquid transfers (aerosol-barrier pipette tips recommended).
- Keep the kit away from any source of contaminating nucleic acids, especially amplified nucleic acid.
- The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.
- All equipment and consumables for a particular operation should be kept in the area where that operation is done and should not be moved between separated areas. Gloves should be removed and disposed of before leaving one area to proceed to the next. Lab coats should be specific to each area and never be worn outside the area.
- The work should flow in one direction, beginning in the extraction area followed by the chosen downstream application areas.

### Performance Data

**Limit of Detection (LoD) - Analytical Sensitivity Study:** To determine the limit of detections (LoD), a dilution series of the pathogen was prepared to give the final concentrations of 2430, 810, 270, 90 and 30 CFU/mL by spiking urine samples collected from negative individuals to mimic clinical specimens. Pathogen DNA was purified using RevoDx Pathogen DNA/RNA Purification Kit. Each dilution was tested in 24 replicates. The Limit of Detection (LoD) value was calculated by probit analysis. The Limit of Detection (LoD) value was 88 CFU/mL. This LoD value was confirmed by testing an additional 20 replicates spiked at 88 CFU/mL. All 20 replicates produced the positive results for each target, and the LoD was therefore confirmed to be 88 CFU/mL.

### Inclusivity:

An *in silico* inclusivity analysis of the RevoDx Mycoplasma Genitalium qPCR Kit primers and probes was performed for the sequences of each genotype available from NCBI databases. The alignments demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available genotype sequences from the National Center for Biotechnology Information (NCBI) databases/databanks.

### Cross Reactivity:

Cross-reactivity of the RevoDx Mycoplasma Genitalium qPCR Kit was evaluated using both *in silico* analysis and by wet testing. The *in silico* analysis of the RevoDx Mycoplasma Genitalium qPCR Kit primers and probes against the sequences of 26 pathogens showed the kit would be specific to the specific targets and not cross-react with these pathogens. The 25 pathogens listed below were wet tested with the RevoDx Mycoplasma Genitalium qPCR Kit for cross-reactivity. No false positive results were observed.

The results from the cross-reactivity, both *in silico* and wet testing, are summarized below.

### *in silico* Cross Reactivity Analysis

Organism	Result
Bacillus subtilis	No homology
Chlamydia pneumoniae	No homology
Haemophilus influenzae	No homology
Legionella pneumophila	No homology
Mycobacterium tuberculosis	No homology
Streptococcus salivarius	No homology
Streptococcus pyogenes	No homology
Bordetella pertussis	No homology
Mycoplasma pneumoniae	No homology
Pneumocystis jirovecii (PJP)	No homology
Enterococcus dispar	No homology
Listeria monocytogenes	No homology
Neisseria meningitidis	No homology
Proteus spp.	No homology
Saccharomyces cerevisiae	No homology
Schizosaccharomyces pombe	No homology
Aspergillus niger	No homology
Salmonella spp.	No homology
Serratia marcescens	No homology
Parainfluenza virus 1-4	No homology
Influenza A & B	No homology
Enterovirus (e.g. EV68)	No homology
Respiratory syncytial virus	No homology
Rhinovirus	No homology
Adenovirus (e.g. C1 Ad. 71)	No homology
Human Metapneumovirus (hMPV)	No homology

## Wet Tested Cross Reactivity Analysis

Organism	Source	Concentration	Result
<i>Chlamydia pneumoniae</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Hemophilus influenzae</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Legionella pneumophila</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Mycobacterium tuberculosis</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Streptococcus pyogenes</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Bordetella pertussis</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Mycoplasma pneumoniae</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Pneumocystis jirovecii</i> (PJP)	Clinical specimen	no unitage assigned	Not Detected
<i>Enterococcus dispar</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Listeria monocytogenes</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Neisseria meningitidis</i>	Clinical specimen	no unitage assigned	Not Detected
<i>Aspergillus niger</i>	Clinical specimen	no unitage assigned	Not Detected
Human coronavirus (229E)	NIBSC (Cat. No: 09/132)	no unitage assigned	Not Detected
Rhinovirus	NIBSC (Cat. No: 08/324)	no unitage assigned	Not Detected
Human Adenovirus	NIBSC (Cat. No: 16/324)	2.9*10 <sup>7</sup> IU/ml	Not Detected
Influenza Virus (A/Christchurch/1/2003, H1N1)	NIBSC (Cat. No: 07/296)	no unitage assigned	Not Detected
Influenza Virus (A/Wyoming/9/2003, H3N2)	NIBSC (Cat. No: 07/298)	no unitage assigned	Not Detected
Influenza Virus (B/Jiangsu/10/2003)	NIBSC (Cat. No: 07/300)	no unitage assigned	Not Detected
Human Immunodeficiency Virus 1 (HIV-1)	NIBSC (Cat. No: 16/194)	1.25*10 <sup>6</sup> IU/ml	Not Detected
Human Immunodeficiency Virus 2 (HIV-2)	NIBSC (Cat. No: 16/296)	2.8*10 <sup>6</sup> IU/ml	Not Detected
Human Respiratory syncytial virus A2	NIBSC (Cat. No: 08/120)	no unitage assigned	Not Detected
Parainfluenza virus type 1	NIBSC (Cat. No: 08/176)	no unitage assigned	Not Detected
Parainfluenza virus type 2	NIBSC (Cat. No: 08/178)	no unitage assigned	Not Detected
Parainfluenza virus type 3	NIBSC (Cat. No: 08/118)	no unitage assigned	Not Detected
Parainfluenza virus type 4	NIBSC (Cat. No: 08/180)	no unitage assigned	Not Detected

Table 3: Amplification program

Program Name	Cycles	Program
Hot Start	1	95°C, 2 min
Amplification*	40	95°C, 10 sec
		60°C, 20 sec

\* Fluorogenic data should be collected at 60°C; FAM and HEX channels should be chosen

5. Fluorogenic data is collected at 60°C. FAM and HEX channels should be selected.

6. Start run.

7. To program and analyze the results, refer to the User Manual of the instrument concerned.

## Data Analysis

In order to evaluate the assay, the Ct value of Positive Control in the FAM channel must be equal to 25±4, and Negative Control in all channels must be negative. Otherwise, the experiment should be repeated.

The results can be interpreted as:

Signal in FAM channel	Signal in HEX channel	Interpretation
+	+/-	Mycoplasma Genitalium DNA positive
-	+	The result is valid. Mycoplasma Genitalium DNA is not detected
-	-	The result is invalid. This sample should be re-tested.

## Ordering Information

Product Name	Package	Cat. No.
RevoDx Mycoplasma Genitalium qPCR Kit	100 tests	IP202243-100
RevoDx Mycoplasma Genitalium qPCR Kit	500 tests	IP202243-500

## Additional Materials Required

- RevoDx Pathogen DNA/RNA Purification Kit (Cat. No: IP202302; idil biotech, Turkey) or RevoDx Magnetic Pathogen DNA/RNA Purification Kit (Cat. No: IP202303; idil biotech, Turkey) or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent (Cat. No: IP202319; idil biotech, Turkey)
- Real-Time PCR Detection System,
- Suitable protection (protective lab coat, disposable gloves, protective goggles, etc.)
- Micropipettes (0.5 µl – 1000 µl),
- DNase/RNase-free micropipette tips with filters,
- DNase/RNase-free 1.5 ml microcentrifuge tubes,
- Vortex mixer,
- Desktop microcentrifuge for PCR plates/strip tubes,
- PCR Workstation,
- Real-Time PCR reaction tubes or plates,

## Sample Preparation

This kit has been validated for use with urine samples or urogenital swabs or cervical swabs. Furthermore, all nucleic acid samples that are suitable for qPCR assays can be used with the Kit. Clinical specimens should be treated as potentially infectious; and the precautions are recommended during sample collection and handling.

Clinicians (including healthcare assistants, nurses, doctors and professionals allied to medicine) have the responsibility of using the correct procedure during the collection and safe transportation of samples to the laboratory. The validity of test results largely depends on good practice in the 'pre-test' stage and it is essential that documentation is accurate and comprehensive.

After collecting, do not store the specimens at room temperature for longer than 4 hours. Transportation of the specimens must conform to country or local regulations.

## Protocol

**DNA Purification** RevoDx Pathogen DNA/RNA Purification Kit or RevoDx Magnetic Pathogen DNA/RNA Purification Kit or DirEXT OneStep Pathogen DNA/RNA Extraction Reagent should be used for DNA extraction from clinical specimens. Using other purification kits may adversely affect the performance characteristics of the kit. Please follow the manufacturer's instructions as stated in the kit manual. The operations should ideally be done in three separate areas. (i.e. for DNA/RNA purification, PCR setup, amplification) to prevent contamination.

**Internal Control** An internal control targeting RNase P is needed to verify that nucleic acid is present in every sample and is used for every sample processed. This also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

**Positive Control** To be able to evaluate the experiment, the Ct values of Positive Control should be equal to 25 ± 4, otherwise, it indicates a problem.

## PCR Protocol

1. Thaw all components at room temperature except M. Genitalium RM 2. Put M. Genitalium RM 2 on ice. Mix each component thoroughly, then centrifuge briefly before use. Transfer all the reagents onto ice or cooling block.

2. The final volume of Master Mix is obtained by multiplying single reaction volumes of M. Genitalium RM 1 and M. Genitalium RM 2 by the total sample size. When calculating the total sample size, the number of negative/positive controls and the clinical samples should be taken into consideration. For possible pipetting errors, it is recommended to add an extra sample to the total sample size.

3. To prepare master mix, add 14 µl of M. Genitalium RM 1 and 1 µl of M. Genitalium RM 2 for each sample to the master mix tube. Vortex the tube and spin down briefly in a microcentrifuge. Add 15 µl of Master Mix into Real-Time PCR reaction tubes or capillaries for each sample. Add 5 µl DNA of each sample, negative control and positive control into the tubes. Spin down briefly.

4. Enter cycling conditions for Real-Time PCR Detection System: 95°C for 2 min, 1 cycle; 95°C for 10 sec, 60°C for 20 sec, 40 cycles (Table 3). Enter 20 µl as sample volume.