Product Manual

AMPIPROBE® *Ureaplasma spp. / M. genitalium / M. hominis* Assay Kit

Catalog #: ENZ-GEN209-0100

100 Assays

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

DESCRIPTION

The AMPIPROBE® *Ureaplasma spp. / M. genitalium / M. hominis* (UMM) Assay is a real-time polymerase chain reaction (qPCR) assay for the qualitative detection of *Ureaplasma spp. (Ureaplasma parvum* and *Ureaplasma urealyticum), Mycoplasma genitalium,* and *Mycoplasma hominis* DNA. The kit uses the AMPIPROBE® assay platform which takes advantage of paired fluorophore- and quencher-labeled primers specific for each of the target species as well as an internal control. The kit contains all reagents necessary for PCR-based detection of *Ureaplasma spp., Mycoplasma genitalium,* and *Mycoplasma hominis* DNA. A positive PCR control consisting of a mixture of the target templates and a negative PCR control that results negative for UMM, but positive for the internal control (human ß-globin). Please read the complete kit insert before performing this assay.

INTRODUCTION

Ureaplasma and *Mycoplasma*, which belong to the same Mycoplasmataceae family and Mollicutes class, are the smallest self-replicating organisms and are characterized by their lack of a cell wall.[1] These characteristics and limited biosynthetic capabilities contribute to the parasitic nature of *Ureaplasma* and *Mycoplasma*.[2]

The class of pathogens is present in healthy individuals but has been associated with many adverse conditions affecting the reproductive tract.[2] *Mycoplasma genitalium* is associated with urethritis, cervical inflammation, and pelvic inflammatory disease.[3] *M. hominis* is often present concurrently with *Ureaplasma* species and is associated with a variety of conditions ranging from pelvic inflammatory diseases, chorioamnionitis, postpartum endometritis bacterial vaginosis, arthritis, osteoarthritis, wound infections, and several conditions in neonates.[4]

Ureaplasma and *Mycoplasma*, especially in combination with other conditions such as bacterial vaginosis or cervical incompetence, have been associated with adverse pregnancy outcomes, such as chorioamnionitis, spontaneous preterm labor and preterm premature rupture of membranes.[5]

The AMPIPROBE® UMM Assay provides rapid and accurate results for the qualitative detection of *Ureaplasma spp. (Ureaplasma parvum* and *Ureaplasma urealyticum), Mycoplasma genitalium,* and *Mycoplasma hominis* DNA in a user-supplied sample of interest.

AMPIPROBE® TECHNOLOGY

Enzo's AMPIPROBE® technology incorporates probe detection technology in primer design. It employs a combination of fluorescent reporter-labeled primers and quencher-labeled primers to amplify DNA, akin to traditional PCR. When free in solution, fluorescent primers generate a signal. However, as the primers are incorporated into amplified DNA, the quencher and the fluorophore are brought within close proximity and exhibit Förster resonance energy transfer (FRET). This causes a logarithmic decay of signal with respect to the number of PCR amplification cycles. The cycle number at which fluorescence signal drops below a defined threshold, is indicative of the amount of target nucleic acid present in the sample.

Enzo's AMPIPROBE® Assay kits provide the following benefits:

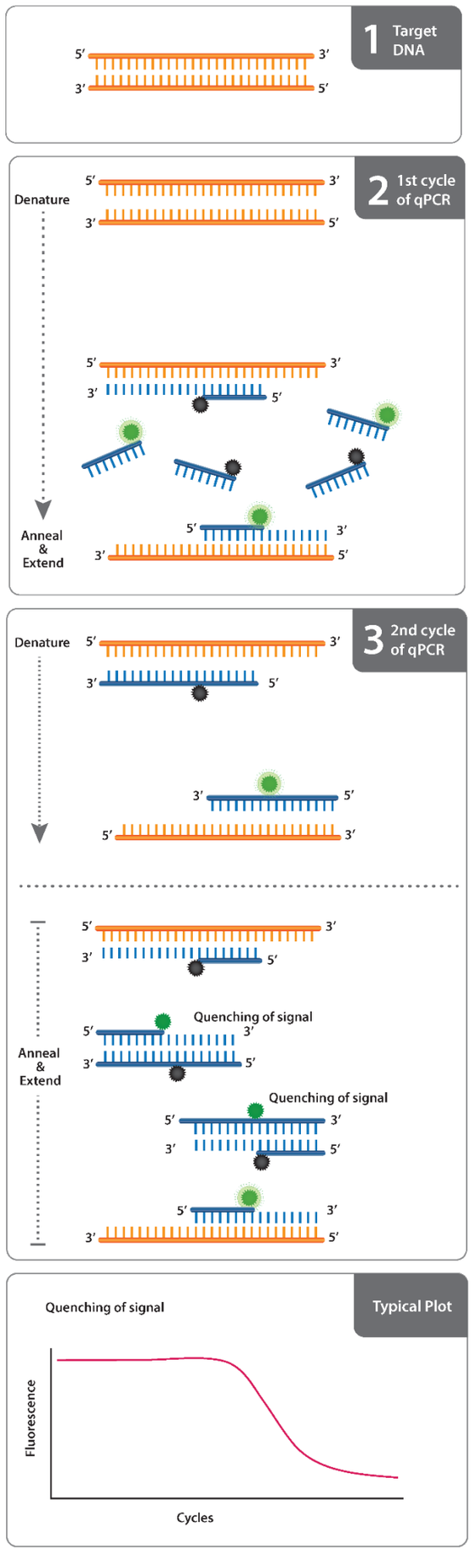
- High sensitivity
- Low sample input
- Adaptable to multiplexing
- Compatible with open qPCR instruments

PRINCIPLE OF AMPIPROBE® UMM ASSAY

The AMPIPROBE® UMM Assay is a qualitative real-time PCR assay for the detection of *Ureaplasma spp. (Ureaplasma parvum* and *Ureaplasma urealyticum), Mycoplasma genitalium, Mycoplasma hominis* genomic DNA. The nucleotide sequences of the PCR primers have been optimized to target sequences within species-specific regions of the target genome. The UMM primers have been designed using AMPIPROBE® technology which incorporates reporter and quencher dyes into the primers. Target detection is based on fluorescence decay when successive rounds of amplification bring fluorophore and quencher-labeled primer pairs in close proximity resulting in FRET. Loss of fluorescence below a defined threshold in a particular channel indicates sample positivity for the corresponding UMM species.

The AMPIPROBE® UMM Primer Mix includes primers for a ubiquitously conserved human housekeeping gene (human ß-globin). Successful amplification of the internal control serves as an indicator of sample adequacy, extraction efficiency and successful amplification in each individual sample.

AMPIPROBE® TECHNOLOGY SCHEMATIC



SAFETY WARNINGS & PRECAUTIONS

Avoid freeze / thaw cycles

Handle with care

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- Kit components should be stored at or below -20°C. Care should be taken to limit the number of freeze-thaw cycles.
- The AMPIPROBE® UMM Primer Mix contains fluorescently labeled primers. To avoid photobelaching, protect from prolonged exposure to light.
- Some components of this kit may contain hazardous substances. Reagents can be harmful if ingested or absorbed through the skin and may cause irritation to the eyes. Reagents should be treated as possible mutagens and should be handled with care and disposed of properly.
- Practice aseptic technique when handling reagents to avoid introduction of contaminants that might interefere with assay interpretation.
- The use of screw-cap tubes and barrier pipette tips is strongly encouraged to prevent samples and reagents from becoming aerosolized which might lead to contamination.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas. Any biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

MATERIALS SUPPLIED

Reagent	Quantity
AMPIGENE® HS Taq DNA Polymerase	100 µL
AMPIGENE® dNTP Mix	50 µL
AMPIPROBE® 5X Assay Buffer	500 µL
AMPIPROBE® UMM Primer Mix	500 µL
UMM Positive PCR Control	100 µL
Negative PCR Control	100 µL
Nuclease-free Water	1 mL

STORAGE

All components of this kit are stable at -20°C until the kit's expiration date.

OTHER MATERIALS NEEDED

1. Vortex mixer
2. Mini centrifuge
3. Calibrated pipettes capable of delivering volumes between 1 and 1000 µL
4. DNase/RNase free barrier pipette tips
5. DNase/RNase free 1.5 mL Screw Cap Micro tube (for preparation of master mix)
6. qPCR instrument and compatible accessories

PROCEDURAL NOTES

Do not mix components from different kit lots or use reagents beyond the kit expiration date.

SAMPLE COMPATIBILITY

Sample types and DNA extraction methods should be evaluated for compatibility with the AMPIPROBE® UMM Assay Kit as part of the user's validation process.

REAGENT PREPARATION

1. Pre-cool Loading Block at 4°C for at least one hour prior to setup.
2. Thaw the following kit reagents at room temperature (15-25ºC): Nuclease-free Water, AMPIGENE® dNTP Mix, AMPIPROBE® 5X Assay Buffer, AMPIPROBE® UMM Primer Mix, UMM Positive PCR Control, and Negative PCR Control.

   Once thawed, vortex the reagents to mix. Spin briefly to collect the contents at the bottom of tubes. Keep the reagents on ice until ready to use. Protect the AMPIPROBE® UMM Primer Mix from prolonged exposure to light.

   Note: Some precipitation may occur in the AMPIPROBE® 5X Assay Buffer. Vortex and warm (37°C) vial to dissolve precipitate prior to use
3. Remove the AMPIGENE® HS Taq DNA Polymerase from storage and briefly spin to collect contents at the bottom of tube. **Do not vortex**. Keep tube on ice until ready to use.

PCR SETUP

The procedure described below uses the Qiagen Rotor-Gene Q instrument, but other real-time PCR machines capable of detecting fluorescence quenching may also be used. Refer to the manufacturer's instrument manual for details regarding operation of the PCR machine.

For each assay run, include the following controls: (a) UMM Positive PCR Control, (b) Negative PCR Control, and (c) No Template Control (not included in kit). Depending upon the user's requirements, additional controls may be needed.

1. Immediately prior to use, prepare sufficient **PCR Master Mix** for the DNA samples and controls in a labeled, DNase/RNase free screw-cap tube, according to the volumes given in Table 1. Add the reagents to the tube in the order listed. Include sufficient volume for 2 extra reactions to allow for any pipetting variance.

The **PCR Master Mix** contains all of the components needed for the reaction except the DNA template (sample).

2. Thoroughly mix the **PCR Master Mix** by either inverting the tube or by pipetting up and down until swirls are no longer visible (10-20 times). **Do not vortex**. Centrifuge briefly to bring contents to the bottom of the tube. Avoid prolonged exposure to light.
3. Dispense 15 µL of **PCR Master Mix** into 0.1 mL PCR tubes, taking care to deliver the solution to the bottom of the tube.
4. Add 10 µL sample or control to the PCR tube and mix by pipetting up and down at least 3 times.
5. Cap the tubes and visually inspect to confirm a tight seal and correct volume.
6. Place the tubes in the thermal cycler and run the method. See **PCR RUN PROFILE** section for the method details.

Table 1. Preparation of PCR Master Mix

Reagent	Volume per reaction	Total volume required [a,b]
Nuclease-free Water	3.5 µL	3.5 µL x (N+2)
AMPIPROBE® 5X Assay Buffer	5 µL	5 µL x (N+2)
AMPIPROBE® dNTP Mix	0.5 µL	0.5 µL x (N+2)
AMPIPROBE® UMM Primer Mix	5.0 µL	5.0 µL x (N+2)
AMPIGENE® HS Taq DNA Polymerase	1 µL	1 µL x (N+2)
TOTAL	**15 µL**	**15 µL x (N+2)**

[a] *Total volume required for **N + 2** reactions, where **N** is the number of samples plus controls. When preparing the Master Mix, prepare enough for 2 extra reactions to allow for any pipetting variance.*

[b] *If the number of samples plus controls exceeds 24, more than 2 extra reaction volumes may be needed.*

PCR RUN PROFILE

Create a temperature profile on your PCR instrument as indicated in Table 2. The method described has been verified using a Qiagen Rotor-Gene Q instrument. Use of other instruments may require minor modifications.

Table 2. PCR Cycling Parameters

Step	Parameter	Temp [°C]	Cycles	Hold [mm:ss]
Hold	Enzyme Activation	95°	1	3:00
Cycling	Amplification	95°	40	00:10
		67°		00:30 (Acquiring to Cycling A on Green, Yellow, Orange, Red)

Fluorescence is detected in the **FAM**/Green, **HEX**/Yellow, **ROX**/Orange and **Cy5**/Red channels at the end of the 2nd segment of the cycling step (67°C).

ADDITIONAL INSTRUMENT SETTINGS

The settings described in Table 3 are recommended for use with the Qiagen Rotor-Gene Q instrument. Perform gain optimization on a tube corresponding to No Template Control and set the instrument to perform optimization before first acquisition.

Table 3. Gain Optimization Settings

Channel	Min Reading	Max Reading	Min Gain	Max Gain
FAM/Green	70Fl	80Fl	-10	10
HEX/Yellow	70Fl	80Fl	-10	10
ROX/Orange	70Fl	80Fl	-10	10
Cy5/Red	70Fl	80Fl	-10	10

DATA ANALYSIS

Fluorescent signal intensity is detected in four channels:

- The signal for *Ureaplasma spp.* amplification is detected in the **FAM**/Green channel.
- The signal for *M. genitalium* amplification is detected in the **HEX**/Yellow channel.
- The signal for *M. hominis* amplification is detected in the **ROX**/Orange channel.
- The signal for Internal Control amplification is detected in the **Cy5**/Red channel.

Results are interpreted using the instrument software by determining the cycle number, **Ct**, corresponding to the point where fluorescence signal drops below a defined threshold.

Using the Rotor-Gene Q software, the data for each of the four channels must be analyzed separately. For all four channels convert the data to display on a linear scale, select “Dynamic Tube” and ensure that “Slope Correct” is OFF. The settings recommended in Table 4 can be used to generate **Ct** values.

Table 4. Recommended Data Analysis Parameters

Channel	Ignore First	Threshold
FAM/Green	10	-0.03
HEX/Yellow	10	-0.05
ROX/Orange	10	-0.08
Cy5/Red	10	-0.05

INTERPRETATION OF RESULTS

- The generation of a **Ct** value using the above analysis parameters is indicative of a positive result.
- The absence of a **Ct** value when the above analysis parameters are applied is indicative of a negative result.
- The UMM Positive PCR control must produce a positive result in all channels (see Table 5). If this condition is not met, the entire run is invalid and must be repeated.
- The Negative PCR control must produce negative results in the green, yellow and orange channels AND a positive result in the red channel (see Table 5). If these conditions are not met, the entire run is invalid and must be repeated.
- If included in the assay run, any No Template Control must test negative in the green, yellow and orange channels. If amplification is observed in the red channel the **Ct** must be >35 (see Table 5).
- For any individual samples of human origin there must be a positive result in the red channel with **Ct** < 35. If this condition is not met, results for the individual sample are considered invalid and the sample should be rerun.

Table 5. Expected Results for Assay Controls.

Channel	UMM Positive PCR Control	Negative PCR Control	No Template Control
FAM/Green	POS	NEG	NEG
HEX/Yellow	POS	NEG	NEG
ROX/Orange	POS	NEG	NEG
Cy5/Red	POS	POS	NEG or Ct >35

PERFORMANCE CHARACTERISTICS

Overall assay detection sensitivity will vary based on the methods used for nucleic acid extraction and should be verified independently.

Analytical sensitivity of the PCR reaction, based on results obtained using plasmid controls, is estimated to be in the range of 1 to 50 target copies per reaction.

FREQUENTLY ASKED QUESTIONS

Can this assay be run on an ABI 7500?

Most thermal cyclers can be used, provided the software is equipped to detect fluorescence decay. However, the PCR method profile and subsequent data analysis parameters may require instrument-specific modification. The software accompanying the ABI 7500 instrument is not capable of generating Ct values for a fluorescence quenching assay and although the assay can be run, data must be analyzed independently.

What is the composition of the UMM Positive PCR Control and the Negative PCR Control?

The PCR controls supplied with the AMPIPROBE® UMM Assay Kit are composed of linearized plasmids containing the specific sequences targeted by the primers in this assay. The amount of target in each mixture is formulated to produce a positive result falling within the dynamic range of the assay when it is carried out according to the procedure described in this user manual.

REFERENCES

1. Molecular Biology and Pathogenicity of Mycoplasmas. S. Razin, et al.; Microbiol Mol Biol Rev. 4,1094, (1998)
2. Molecular Methods for the Detection of Mycoplasma and Ureaplasma Infections in Humans. K. Waites, et al. J Mol Diagn. 5, 437, (2012)
3. Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases. J. Jensen, et al.; J Eur Acad Dermatol Venereol. 1, 1, (2004)
4. Mycoplasmas and ureaplasmas as neonatal pathogens. K. Waites, et al.; Clin Microbiol Rev. 18, 757 (2005).
5. Ureaplasma urealyticum, Mycoplasma hominis and adverse pregnancy outcomes. R. Capoccia, et al.; Curr. Opin. Infect. Dis. 26, 231, (2013)

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