

Performance Characteristics

1. Limit of Detection: 1×10^3 copies/mL.














2. Analysis specificity:

No cross-reactivity were observed with *Lactobacillus fermentum*, *Bacteroides fragilis*, *Bacteroides melaninogenicus*, *Lactobacillus crispatus*, *Candida glabrata*, *Candida tropicalis*, *Gardnrella vaginalis*, *Canidia albicans*, *Lactobacillus gasseri*, *Lactobacillus rhamnosus*, *Lactobacillus iners*, *Lactobacillus acidophilus*, *Lactobacillus jensenii*, *Prevotella bivia*, *Atopobium vaginae*, *Bifidobacteriaceae*, *Mobiluncus*, *Treponema pallidum*, *Trichomonas vaginalis*, *Human papillomavirus*, *Human immunodeficiency virus*, *Ureaplasma Urealyticum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *Mycoplasma hominis*.

Precautions

1. This product is only suitable for in vitro diagnostic testing, and the experimenter should have received professional training and obtained the relevant work permit.
2. It shall be implemented strictly in accordance with the management norms of gene amplification laboratory promulgated by the relevant competent authorities. The laboratory is divided into three areas for operation (reagent preparation area, sample preparation area and amplification area), items in each area shall not be cross-used, and special instrument and equipment shall be used in each area.
3. The performance characteristics of this test kit for the sample types listed in the section "Intended use" have been identified. The performance of this test kit for other sample types has not been evaluated
4. The tip with filter and centrifuge tube used in the experiment should be autoclaved, and without DNase and RNase. After use, it is directly driven into the disposal bottle containing 1% sodium hypochlorite.
5. All clinical samples should be treated as infectious substances, operation and disposal shall comply with relevant regulations.

Symbols Meaning

	Do not re-use		Keep away from sunlight
	Keep dry		Consult instructions for use
	Caution		Do not use if package is damaged
	Non-sterile		In vitro diagnostic medical device
	Manufacturer		Batch Code
	Manufacture Date		Symbol for "USE BY"
	Temperature limit(2-8°C)		

Basic Information



Manufacturer: Wuxi Techstar Technology Co.,Ltd

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Eleven Urogenital Pathogens Nucleic Acid Detection Kit (Fluorescent PCR)

For Research Use Only

Name	Eleven Urogenital Pathogens Nucleic Acid Detection Kit (Fluorescent PCR)	
REF	SC540012	SC540022
Specification	48 reactions	96 reactions

Intend Use

This product is used for the qualitative detection of the nucleic acids of following pathogens in the vaginal swab or urine sample: *Klebsiella aerogenes*, *Morganella Morganii*, *Streptococcus Agalactiae*, *Enterococcus Faecalis*, *Proteus Mirabilis*, *Mycobacterium Avium*, *Escherichia Coli*, *Citrobacter spp.*(*Citrobacter Freundi*/*Citrobacter Werkmanii*/*Citrobacter Portucalensis*/*Citrobacter Youngae*/*Citrobacter Cronae*/*Citrobacter Braaki*/*Citrobacter Arsenatis*/*Citrobacter Malonaticus*/*Citrobacter Koseri*), *Klebsiella Pneumoniae*, *Pseudomonas Aeruginosa* and *Staphylococcus Aureus*.

Principle

This product uses Real-Time PCR technology to design specific primers and probes for 11 pathogens. Each test was divided into 3 tubes as shown in the following table. During the amplification process, when the probe is complete, because the 3' quenched group is close to the 5' reporter group, the fluorescence emitted by the reporter group is absorbed by the quenched group, and no fluorescence signal is emitted. When the nucleic acid of the target was contained in the reaction system, the fluorescent probe bound to the template during primer extension was severed by Taq enzyme (5'-3' exonuclease activity), and the reporter group was separated from the quenched group to produce fluorescence signal. The real-time amplification curve can be plotted by fluorescent quantitative PCR instrument automatically according to the detected fluorescence signal. The assay contains an endogenous internal control (CY5 sign in tube C) for monitor specimen collection, nucleic acid extraction and PCR.

	Tube A	Tube B	Tube C
FAM	Streptococcus Agalactiae	Mycobacterium Avium	Staphylococcus Aureus
HEX/VIC	Morganella Morganii	Escherichia Coli	Klebsiella Pneumoniae
ROX	Klebsiella aerogenes	Proteus Mirabilis	Pseudomonas Aeruginosa
CY5	Enterococcus Faecalis	Citrobacter spp.	Internal Control

Materials Provided

Constituent	Component	48 reactions/kit
Dilution Buffer	Nuclease-free water	2 mL/vial, 2 vial
EUP Mix A	Primer, Probe, dNTPs, Enzyme	1 vial
EUP Mix B	Primer, Probe, dNTPs, Enzyme	1 vial
EUP Mix C	Primer, Probe, dNTPs, Enzyme	1 vial
EUP PC	Plasmid containing the target gene	1 vial
EUP NC	Nuclease-free water	500 µl/vial, 1 vial

Note: The components in different batch are not interchangeable.

Materials and Equipment Required but Not Provided

- Applied Biosystems™ Real-Time PCR System 7500. Alternatively, Roche LightCycler® 480 System, or QuantStudio 5 Real-Time PCR System.
- QIAamp UCP Pathogen Mini Kit manually (cat. # 50214, Qiagen)
- Vortex mixer.
- Microcentrifuge.
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL).
- Racks for 1.5 mL microcentrifuge tubes.
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach).
- Disposable powder-free gloves and surgical gowns.
- 1.5 mL microcentrifuge tubes (DNase/RNase free).
- 96-well 0.2 mL PCR reaction plates or appropriate optical reaction tube.
- Nuclease-free water

Storage Conditions and Expiration Period

Store at 2-8 °C away from light for 12 months or 20-25 °C away from light for 6 months.

Date of manufacture: see label

Expiration Period: See label

Applicable Instrument

ABI7500 - ABI QuantStudio 5 and Roche LightCycler480

Specimen Collection and Preparation

- Types of sample suitable for this product: vaginal swab, urine sample.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test. Improper collection, storage, or transport of specimens may lead to false negative results.
- The specimen may be tested immediately after collection, or it may be stored at 2-8 °C for up to 72 hours before testing. If a delay in testing or shipping is expected, the specimen may be stored at -18 °C for no longer than 1 week or at -70 °C for no longer than 6 months. Avoid repeated freeze-thaw cycles.
- The specimen should be shipped in low temperature conditions using dry ice or an ice bag.

Laboratory Procedures

1. Sample processing

DNA should be collected from fresh a specimen to ensure suitable DNA quality and quantity. DNA should be extracted using the QIAamp UCP Pathogen Mini Kit manually (cat. # 50214, Qiagen) according to the manufacturer's Instruction of Use. Following extraction, the DNA should be used immediately or stored at -20 °C for use later.

2. EUP PC processing

The EUP PC was centrifuged at 10,000 rpm for 1 minutes, 100 µL Nuclease-free water was added to the PC and mix by pipetting.

⚠ The remaining EUP PC must be stored at under -18 °C immediately, and it should be used in 20 days, repeated freezing and thawing times should not exceed 5 times.

3. Master Mix processing

980 µL Dilution Buffer was added to EUP Mix A, EUP Mix B and EUP Mix C respectively for re-dissolution, and was completely mixed with tip.

⚠ The remaining EUP Mix A, EUP Mix B and EUP Mix C must be stored at under -18 °C immediately, and it should be used in 20 days, repeated freezing and thawing times should not exceed 5 times.

4. PCR reagent processing

4.1 Prepare 96-well plates or appropriate optical reaction tube for real-time PCR based on the estimated number of reactions, three tubes are required for each sample.

4.2 Pipette 20 µL of EUP Mix A, EUP Mix B and EUP Mix C into each three wells. Cover and transfer the plate into "sample processing area".

5. Sample DNA Addition

5.1 Add 5µL extracted sample DNA, EUP PC and EUP NC into each three wells(EUP Mix A, EUP Mix B and EUP Mix C) respectively.

5.2 Close the 96-well reaction plate with appropriate lids or optical adhesive film, mix evenly upside down and short spin at 3000rpm.

6. Testing

6.1 The PCR reaction tube was placed in the PCR apparatus.

6.2 Double-click 7500 software and select Start>>All Programs>>Applied Biosystems>>7500 Software.

Click New Experiment to enter Experiment menu. In the Experiment Properties screen, enter identifying information for the experiment; you can leave other fields empty.

Select 7500 (96 Wells); Quantitation-Standard Curve (for the experiment type); TaqMan Reagents (for reagent); and Standard (for ramp speed).

Click Plate Setup, in the Targets screen, under the tab Define Targets and Samples, set Target 1 with FAM reporter, Target 2 with VIC/HEX reporter, Target 3 with ROX reporter, and Target 4 with CY5 reporter. Quencher Dye all set: None. Define samples according to the samples in this experiment.

Click Assign Targets and Samples tab, in the target(s) screen, for the three tubes(Mix A/B/C of a test), select target 1, target 2, target 3 and target 4. In the Samples screen, select samples and controls to include in the reaction plate in corresponding well, and select the sample/target reactions to set up. Select None for passive reference.

Click Run Method. On the Run Method screen, select either the Graphical View tab (default) or the Tabular View to edit the run method. Make sure the thermal profile displays the holding and cycling stages shown below.

Step	Temperature	Time	Fluorescence measured	Cycle
1	95 °C	3min	No	1
2	95 °C	15sec	No	45
3	58 °C	30sec	Yes(FAM, VIC/HEX, ROX, CY5)	

Click Run. In the Run screen, save the experiment. Click START.

After the run completes, unload the instrument and proceed to data analysis.

7. Analysis of result

Click Analysis. In the Amplification Plot screen under Plot Settings tab:

- In the Plot Type drop-down list, select ΔRn vs Cycle (default).
- In the Graph Type drop-down list, select Linear.
- In the Plot Color drop-down list, select Target.

Set the baseline starting point at cycle 3 and ending at cycle 15

Adjust the threshold to be equal to the maximum level of the no-template control curve (i.e., equal to the maximum value of the random noise curve).

Click Analyze. The software analyzes the data with the settings.

Interpretation of Result

1. Quality Control Standards

- EUP NC(Mix A and B): no typical S-type amplification curve or Ct>40 for all channels.
- EUP NC(Mix C): no typical S-type amplification curve or Ct>40 for FAM, HEX (VIC) or ROX channels.
- EUP PC(Mix A, B and C): all the 4 channels showed a typical S-type amplification curve and Ct ≤35.
- Positive control and Negative control are used to monitor the effectiveness of the instrument and test reagents. The test results of all indexes can only be interpreted when the test results meet the conditions in section 1.1, 1.2 and 1.3 at the same time.

2. Result interpretation

Under the premise that Positive control and Negative control meet the requirements, the following analysis is carried out:

- If the Ct values of any of the 11 indicators detected by this product in their respective channel meet the requirements in the below, the indicator is positive, as shown in the following table.

Tube	Channel	Indicators	Positive
Tube A	FAM	Streptococcus Agalactiae	Showed a typical S-type amplification curve and Ct≤38
	HEX/VIC	Morganella Morganii	
	ROX	Klebsiella aerogenes	
	CY5	Enterococcus Faecalis	
Tube B	FAM	Mycobacterium Avium	
	HEX/VIC	Escherichia Coli	
	ROX	Proteus Mirabilis	
Tube C	CY5	Citrobacter spp.	
	FAM	Staphylococcus Aureus	
	HEX/VIC	Klebsiella Pneumoniae	
	ROX	Pseudomonas Aeruginosa	
	CY5	Internal Control	/

If two or more of the above conditions are present in one sample at the same time, it is indicated as multiple infection.

- Negative: the CY5 channel of tube C showed a typical S-type amplification curve and Ct≤38, the other 11 channels had no Ct value(or Ct>38).
- If there is no typical S-type amplification curve in any channel, it indicates that the system is inhibited or misoperated, and the test is invalid. Therefore, the samples should be reexamined.

Limitations

- The results obtained by this kit should be interpreted in conjunction with other laboratory data and clinical data obtained by the doctor. It should not be used alone as a basis for patient management.
- Negative results cannot completely exclude pathogen infection, because a concentration of target gene in the sample lower than the test limit or mutation in target sequence can lead to negative results. Improper sample collection, delivery, and handling, as well as improper test operation and experimental environment, can all lead to false negative or false positive results.
- The samples collected from the patients who used drug therapy may result in false negative results.
- The test nucleic acid may remain in the body for a long period of time, regardless of activity of the pathogen. A positive test result does not necessarily mean that the pathogen is infectious.