1.0 INTENDED USE

The Urinary Tract Infection (UTI) Panel Assay is a qualitative, single-plex, nucleic acid in-vitro amplification assay that detects various urinary tract microbiota in urine samples.

2.0 TEST PRINCIPLE

To test for presence/absence of urinary tract microbiota, the Lioness UTI Panel Assay uses qPCR. The assay involves two main steps: 1) Nucleic acid extraction and 2) amplification/detection of target DNA by real-time PCR. Amplification products are detected via fluorescent dyes during the PCR. The kit contains all reagents necessary to perform amplification and detection for up to 16 tests for all 23 pathogens and extraction control. The test relies on the extraction of high-quality nucleic acid from bacteria and yeast from urine samples.

Taq polymerase is not included in the assay and must be ordered separately. Researchers will need to choose the Taq polymerase properties appropriate for their assay. SeqOnce suggests using SeqOnce Amplification Kit 1 with InhibiTaq HotStart.

The Lioness UTI Panel Assay is for Research Use Only.

3.0 MATERIALS PROVIDED AND STORAGE

3.1. SeqOnce Lioness UTI Panel Assay and SeqOnce Amplification Kit 1 (Optional)

Materials Provided and Storage:
Concentration: 2X InhibiTaq HotStart, Multiplex Master Mix and 5X Primer/Probe Mixes
Storage and Handling: Store at -20˚C upon arrival

Ordering Information: Lioness UTI Panel Assay, 16 Reactions

Item Number	Component	Quantity Received	
LUTI-PA-16	SB-LUTI-PPM-16-PC SB-LUTIPC-100ul-PC	1xPlate 3x100uL	
Component	**Description**	**Volume**	**Storage**
SB-LUTIPC-100ul-PC	Lioness 24 UTI Positive Control	3x100uL	-20°C
SB-LUTI-PPM-16-PC	5X Lioness UTI Panel Primer Probe Mix Plate	1 x 40µL	-20°C

Ordering Information: SeqOnce Amplification Kit 1 (Optional)

Item Number	Component	Quantity Received
SQAK1-200	SB-ITMP-MM-100-PC	2x1mL

3.2. Organism list on UTI Panel and Well Position

Organism	Well on Plate
Acinetobacter baumannii	A1
Klebsiella aerogenes	A2
Enterobacter cloacae	A3
Enterococcus faecium	A4
Escherichia coli	A5
Klebsiella pneumoniae	A6
Proteus vulgaris	A7
Pseudomonas aeruginosa	A8
Streptococcus agalactiae (group B)	A9
Serratia marcescens	A10
Mycoplasma hominis	A11
Ureaplasma urealyticum	A12
Staphylococcus aureus	B1
Staphylococcus saprophyticus	B2
Proteus mirabilis	B3
Enterococcus faecalis	B4
Klebsiella oxytoca	B5
Morganella morganii	B6
Citrobacter freundii	B7
Providencia stuartii	B8
Candida albicans	B9
Candida glabrata	B10
Candida tropicalis	B11
RPP Extraction control	B12

4.0 SUPPLIES/MATERIALS REQUIRED

- qPCR 96-well plate
- qPCR 384-well plate
- qPCR plate sealer
- 20µL pipette tips
- 200µL pipette tips
- 1mL pipette tips
- DNA Extraction Kit (e.g. MagMAX™ DNA Multi-Sample Ultra Kit)
- Positive Microbiota Controls

5.0 EQUIPMENT REQUIRED

- 2.5µL pipette
- 10µL pipette
- 20µL pipette
- 200µL pipette
- 1mL pipette
- Centrifuge, mini
- qPCR machine
- Vortex
- Plate Spinner

6.0 SAFETY PRECAUTIONS

- For Research Use Only.
- Wear disposable gloves, laboratory coats and protective eyewear when handling reagents and samples.
- Thoroughly wash your hands after handling reagents and samples. Do not eat, drink, or smoke in designated work areas.
- Handle all samples as potentially infectious, in accordance with Good Laboratory Practices.
- Chemicals must be handled and disposed of in accordance with Good Laboratory Practices.

7.0 SAMPLE REQUIREMENTS

- Urine sample in a clean collection device.

8.0 QUALITY CONTROL

- A Negative and a Positive Control must be included in each assay run in order to detect potential failure in specimen processing, amplification or detection steps.
- RPP (RNAse P) Extraction Control, which is an extracted internal control, amplified and detected in the same way as target DNA, allowing the control of the extraction procedure and the detection of potential PCR inhibition.

9.0 PROCEDURE

9.1. DNA extraction of samples

Refer to DNA extraction protocol provided with DNA extraction kit (MagMAX™ DNA Multi-Sample Ultra Kit) or equivalent.

9.2. Master Mix Preparation

Add reagents in Table 2 in order shown to the labeled 1.5mL or 2mL Eppendorf tube.

Table 2: Master Mix setup

Lot No.	Exp. Date	Component	Volume per rxn (µL)	Volume for X rxns (µL)
		Primer/Probe Mix	2	
		2X InhibiTaq Multiplex Master Mix	5	

9.3. Plating Samples onto qPCR plates

1. Obtain a 96-well qPCR plate.
2. Pipette 2µL of each primer/probe x number of samples + (2)controls + 10% overage into each appropriate well.
   - Example: for 10 samples add 26.5 µl of primer/probe mix
3. Pipette 5µL of 2X InhibiTaq Master Mix x number of samples + (2)controls + 10% overage into wells with primer/probe.
   - Example: for 10 samples add 66 µl of Master Mix
4. Mix up and down 2-3x, be sure not to make any bubbles.
5. Seal plate and centrifuge briefly to settle reagents.
6. Using multichannel, pipette 7µL from each well of 96-well plate and transfer onto 384 well plate.
7. Pipette 3µL samples into appropriate wells, and negative/positive samples into appropriate wells onto 384-well plate.

8. Pipette up and down 2-3x to mix. Be sure to avoid any bubbles in wells by only pipetting to first stop (to avoid introducing bubbles).
9. Seal plate with optical seal and Spin down plate with plate centrifuge.
10. Place sealed plate in qPCR instrument.

9.4. qPCR Instrument Setup

1. Set up qPCR protocol as shown below:
    a) 95°C for 2 min
    b) 95°C for 3 sec ____ 40 cycles (repeat steps b, c)
    c) 60°C for 30 sec /
2. Select the following dyes:
    a) FAM for pathogens.
    b) Apply all dyes to all wells that are to be tested.
3. Once setup is complete and plate is properly inserted into qPCR instrument, then start run.

10.0 INTERPRETATION OF RESULTS

NOTE: Appropriate cut off for positivity and analysis must be independently established. Guidelines below are for general use only and are not to be used as the sole method of interpretation.

Sample Interpretation

FAM Channel	Analysis
Ct < 40	Organism detected
Ct is >41	Organism not detected

11.0 MANUFACTURING INFORMATION

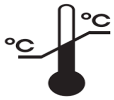
Empirical Bioscience
2007 East Castle Drive, SE
Grand Rapids, MI 49508

12.0 SUPPORT

Phone: +1-626-460-3050

Email: info@seqonce.com

13.0 EXPLANATION OF SYMBOLS

Symbol	Definition
	Lot Number
	Manufacturer
	Date of Manufacture
	Storage & Transport Conditions
	Consult instructions for use
	Use by Date