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NOT FOR IN VITRO DIAGNOSTICS

NOT FDA APPROVED

Lioness UT Microbiota Panel A

Bandit Biosystems — Multiplex qPCR Primer/Probe & Controls Kit for Urinary Tract Microbiota Detection

Bandit BIOSYSTEMS

Description

The **Lioness UT Microbiota Panel A** is a multiplex qPCR primer/probe and controls kit used to detect nucleic acid from specific urinary tract organisms. The panel can be used in multiplex qPCR to detect nucleic acid from bacteria, yeast, and other microorganisms associated with urinary tract infections.

Key Product Features

- Detects nucleic acid from 24 urinary tract microbiota targets
- Optimized for 96-well and 384-well formats at 15 µL reactions
- 8 multiplexed 15X Primer/Probe Mixes included
- 3 UTI Positive Controls included

Important Notices

- May not detect emerging novel mutated strains
- Amplification master mix kit must be purchased separately (PN: 500023)
- Use of alternate master mixes is not supported by Bandit Biosystems

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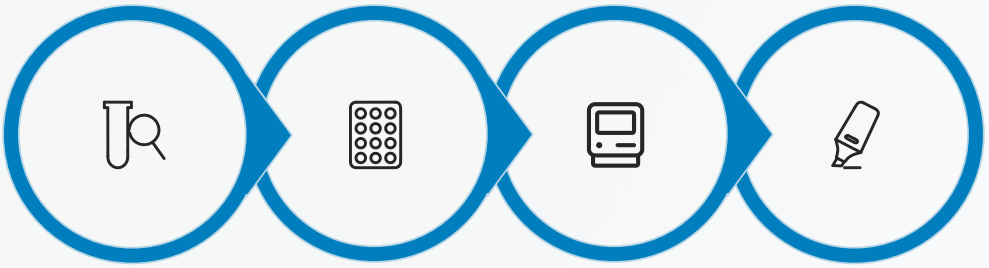
Target Organisms

The panel simultaneously detects nucleic acid from the following 24 urinary tract microbiota targets across bacteria, fungi, and atypical organisms in a single multiplex qPCR workflow:

Target Organism	Target Organism
Acinetobacter baumannii	*Proteus vulgaris*
Staphylococcus aureus	*Citrobacter freundii*
Klebsiella aerogenes	*Pseudomonas aeruginosa*
Enterococcus faecalis	*Staphylococcus saprophyticus*
Enterobacter cloacae	*Streptococcus agalactiae* (grp B)
Klebsiella oxytoca	*Candida glabrata*
Enterococcus faecium	*Candida tropicalis*
Morganella morganii	*Candida parapsilosis*
Escherichia coli	*Candida albicans*
Proteus mirabilis	*Serratia marcescens*
Klebsiella pneumoniae	*Mycoplasma hominis*
Providencia stuartii	*Ureaplasma urealyticum*

Test Principle

The Lioness UT Microbiota Panel A leverages **TaqMan-based multiplex qPCR chemistry** to simultaneously detect multiple urinary tract microbiota targets in a single reaction. The human RNaseP (hRPP) gene is used as an internal sample integrity control.



Sample Extraction | qPCR Plate Setup | qPCR Amplification | Fluorescence Analysis

During amplification, the 5' nuclease activity of Taq polymerase degrades the probe, separating the reporter dye from the quencher and generating a fluorescent signal that increases with each cycle. The DNA polymerase amplifies DNA fragments sharing homology with the primer/probe sets specific to each target organism.

Step 1

Sample Extraction — isolate DNA using a validated extraction method

Step 2a

Master Mix Setup — combine 2X Master Mix, nuclease-free water, and 15X Primer/Probe Mix as described in Master Mix reaction setup table.

Step 2b

Add 4 µL extracted sample, positive control, or NTC to each well containing 11 µL master mix

Steps 3–4

Run qPCR and analyze fluorescence data per channel

Required Instrumentation

Equipment	Specification
qPCR Instrument	Any instrument capable of detection in FAM, HEX, Cy5, and ROX fluorescence channels or similar wavelengths

Reagents, Kit Contents & Storage

The Lioness UT Microbiota Panel A kit includes the primer/probe mixes and positive controls required for multiplex qPCR. Amplification master mix must be purchased separately. The following kit configuration is available:

Kit 500012 — 48 Reactions

Plexes 1–8 (Part Numbers 400071–400078) + UTI Positive Controls 450001, 450002, 450003

Component Details

Part Number	Description	Volume	Storage
400071–400078	15X Individual Primer/Probe Mixes — (8) Multiplexes	(8) × 48 µL	–20°C
450001 450002 450003	UTI Positive Controls — 3 individual control tubes with specific controls in each tube	(3) × 100 µL	–20°C

Recommended But Not Included

Part Number	Description	Volume	Storage
500023	SeqOnce qPCR Multiplex Amplification Kit 3000	(5) × 0.6 mL Master Mix (PN 400084) + (1) × 1 mL Water	–20°C

Store all kit components at –20°C upon arrival. Only one master mix kit (PN: 500023) is required. Use of alternate master mixes is not supported by Bandit Biosystems.

Quality Control

Quality Control Requirements

Include a **Negative Control (Water/NTC)** and **Positive Control** in every assay run

hRPP (hRNAse P) Extraction Control is included in the Primer/Probe mix as an internal DNA control, allowing control of the extraction procedure and detection of potential PCR inhibition

Controls are provided at approximately **~10,000 copies/µL**; dilutions may be made in Tris-EDTA (TE) pH 8.0

Controls are **not intended to be extracted** — add directly to amplification reaction

Controls Provided

Control	Part Number	Targets Included
Infectious Disease Control 1	450001	*Staphylococcus saprophyticus*, *Streptococcus agalactiae*, *Citrobacter freundii*, *Proteus mirabilis*, *Morganella morganii*, *Proteus vulgaris*, *Acinetobacter baumannii*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*
Infectious Disease Control 2	450002	*Enterobacter aerogenes*, *Enterococcus faecium*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Serratia marcescens*
Infectious Disease Control 3	450003	*Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida albicans*, Human RNase P control

PRIMER/PROBE MULTIPLEX CONFIGURATION

The panel is organized into **8 multiplexes (Plexes 1–8)**, each detecting 4 targets across FAM, HEX, Cy5, and ROX fluorescence channels. Select all four dyes for each labeled well.

Plex 1 — Target	**Channel**
Acinetobacter baumannii	FAM
E. coli	HEX
Enterococcus faecalis	Cy5
RNaseP	ROX
Plex 2 — Target	**Channel**
Klebsiella oxytoca	FAM
Klebsiella pneumoniae	HEX
Enterobacter cloacae	Cy5
RNaseP	ROX
Plex 3 — Target	**Channel**
Citrobacter freundii	FAM
Proteus mirabilis	HEX
Morganella morganii	Cy5
RNaseP	ROX
Plex 4 — Target	**Channel**
Klebsiella aerogenes	FAM
Enterococcus faecium	HEX
Ureaplasma urealyticum	Cy5
RNaseP	ROX

Plex 5 — Target	**Channel**
Streptococcus agalactiae	FAM
Staphylococcus saprophyticus	HEX
Serratia marcescens	Cy5
RNaseP	ROX
Plex 6 — Target	**Channel**
Candida parapsilosis	FAM
Candida tropicalis	HEX
Candida glabrata	Cy5
RNaseP	ROX
Plex 7 — Target	**Channel**
Staphylococcus aureus	FAM
Pseudomonas aeruginosa	HEX
Mycoplasma hominis	Cy5
RNaseP	ROX
Plex 8 — Target	**Channel**
Providencia stuartii	FAM
Candida albicans	HEX
Proteus vulgaris	Cy5
RNaseP	ROX

HEX may be replaced by VIC or JOE. ROX may be replaced by Texas Red. Cy5 may be replaced by Quasar 670 if not available on your instrument. Select all four dyes for each labeled well.

Recommended Protocol

Follow these setup instructions carefully to ensure optimal assay performance. All steps should be performed in a clean room or designated setup area. Reaction volumes have been optimized at **15 µL** and may be run in either 96 or 384-well instruments.

01

Extract DNA

Extract DNA from sample per a separate validated protocol as determined by the individual user.

02

Thaw Reagents

Completely thaw Master Mix, nuclease-free water, and all 8 primer/probe mixes on ice (~30 min) or at room temperature (~5 min). Vortex briefly at max speed, then spin down. .

03

Prepare Master Mix

In a clean area, combine per reaction: **7.5 µL** 2X Master Mix (PN: 400084) + **2.5 µL** nuclease-free water + **1 µL** 15X Primer/Probe Mix. Each of the 8 plexes must be prepared separately. Vortex briefly to mix.

04

Dispense & Add Sample

Distribute **11 µL** of master mix to all designated wells. Add **4 µL** of extracted sample DNA, positive control, or NTC to appropriate wells.

05

Seal & Centrifuge

Seal the plate and centrifuge for 30 seconds at 3,000 RPM (or highest available speed) to collect mixed samples.

06

Run qPCR

Place the sealed plate into the qPCR instrument and run per the thermal cycling conditions below. Collect fluorescence during the 59°C annealing/extension phase.

Reaction Setup & Thermal Cycling Conditions

Master Mix Reaction Setup (15 µL Total Volume)

Component	Volume / Reaction	Final Concentration
2X Exmoor Master Mix (PN: 400084)🔄	7.5 µL	1X
Nuclease-Free Water	2.5 µL	N/A
15X Primer/Probe Mix🔄 ❗	1 µL	1X
Extracted Sample / Control / NTC	4 µL	N/A

ℹ️ 🔄 Vortex thoroughly before use.

❗ Prepare each of the 8 plex master mixes separately — do not combine.

Thermal Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
Enzyme Activation	1	1	95°C	2 minutes
Amplification — Denaturation	2	40	95°C	3 seconds
Amplification — Annealing/Extension*	2	40	59°C	30 seconds

ℹ️ *Collect fluorescence during the annealing/extension phase (59°C) on FAM, HEX, Cy5, and ROX channels (or equivalent).

Interpretation of Results

ⓘ Appropriate cut-off values for positivity and analysis must be independently established. The guidelines below are for general use and should not be used as the sole method of interpretation.

Ct Value Interpretation

Ct Value	Result	Interpretation
Ct < 38	+	✅ Nucleic Acid Detected
Ct > 38	–	🔴 Nucleic Acid Not Detected

Causes of False-Negative Results

- Improper sample collection
- Degradation of genetic material during shipping/storage
- Specimen collection after pathogen genetic material can no longer be found in the specimen matrix
- Unvalidated media or assay reagents
- Presence of qPCR inhibitors
- Mutation in pathogen genomes
- Improper vortexing and centrifuging when preparing qPCR reactions
- Expired reagents

Causes of False-Positive Results

- Cross contamination during specimen handling or preparation
- Cross contamination between samples
- Specimen mix-up
- DNA contamination during product handling
- Improper sealing of the PCR plate

Support & Contact

For technical support, ordering inquiries, or to request Safety Data Sheets (SDS), please reach out to the Bandit Biosystems team:

📞 Phone

855-792-1225

✉️ Email

info@bandit.bio

📋 SDS Requests

Safety Data Sheets available on request via phone or email

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