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NOT FOR IN VITRO DIAGNOSTICS

NOT FDA APPROVED

Pongo Antibiotic Resistance Panel A

Bandit Biosystems — Multiplex qPCR Primer/Probe & Controls Kit for Bacterial Antibiotic Resistance Genes

Bandit BIOSYSTEMS

Description

The **Pongo Antibiotic Resistance Panel A** is a multiplex qPCR primer/probe and controls kit used to detect nucleic acid from specific bacterial antibiotic-resistant genes. The panel is intended to be used in conjunction with or reflex to the Lioness UT Microbiota Panel A or Glyphis Wound panel.

Key Product Features

- Detects nucleic acid from 24 bacterial antibiotic resistance gene targets
- Optimized for 96-well and 384-well formats at 15 µL reactions
- 6 multiplexed 15X Primer/Probe Mixes included
- 3 ABR Positive Controls included

Important Notices

- May not detect emerging novel mutated strains
- Master mix must be purchased separately (PN: 500022)
- Use of alternate master mixes is not supported by Bandit Biosystems

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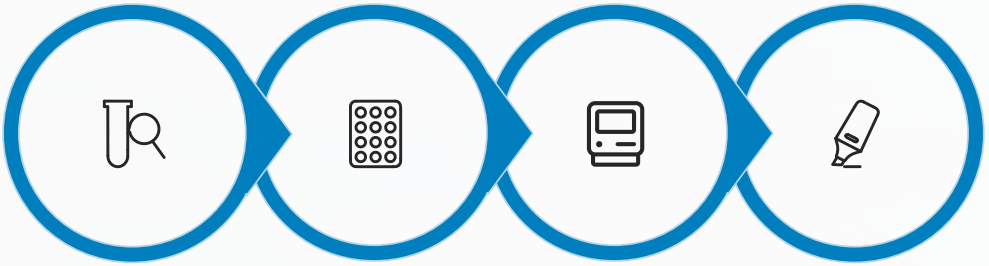
Target Resistance Markers

The panel simultaneously detects the following 24 antibiotic resistance gene targets across multiple resistance categories in a single multiplex qPCR workflow:

Resistance Category — Abbreviation	Resistance Category — Abbreviation
AmpC Resistance Marker — ACT	Carbapenem Resistance Marker — VIM, bla-VIM
AmpC Resistance Marker — AmpC Gene	Carbapenem Resistance Marker — IMP-7, bla-IMP
AmpC Resistance Marker — Fox	ESBL Resistance Marker — SHV
Methicillin Resistance Marker — mecA	ESBL Resistance Marker — TEM
Quinolone/Fluoroquinolone Resistance — qnrB	ESBL Resistance Marker — CTX-M Group 1
Quinolone/Fluoroquinolone Resistance — qnrA	ESBL Resistance Marker — CTX-M Group 2
Quinolone/Fluoroquinolone Resistance — qnrS	ESBL Resistance Marker — CTX-M Group 9
Vancomycin Resistance Marker — vanA1_vanA2	Macrolide Resistance Marker — ErmA
Vancomycin Resistance Marker — vanB	Macrolide Resistance Marker — ErmB
Carbapenem Resistance Marker — NDM-1	Sulfonamide — SUL1
Carbapenem Resistance Marker — KPC, bla-KPC	Sulfonamide — SUL2
Carbapenem Resistance Marker — OXA-48, bla-OXA	Tetracycline — Tet(A)

Test Principle

The Pongo Antibiotic Resistance Panel A leverages **TaqMan-based multiplex qPCR chemistry** to simultaneously detect multiple bacterial antibiotic resistance gene targets in a single reaction.



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 antibiotic resistance gene target.

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 Pongo Antibiotic Resistance 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 500003 — 48 Reactions

Plexes 1–6 (Part Numbers 400022–400027) + ABR Positive Controls 450007, 450008, 450009

Component Details

Part Number	Description	Volume	Storage
400022–400027	15X Individual Primer/Probe Mixes — (6) Multiplexes	(6) × 48 µL	–20°C
450007 450008 450009	ABR 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
500022	SeqOnce qPCR Multiplex Amplification Kit 2400	(4) × 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: 500022) 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

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

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

Controls Provided

Control	Part Number	Targets Included
ABR Positive Control 1	450007	blaACT, mecA, qnrA, qnrB, qnrS, vanA1-A2, vanB, SUL2
ABR Positive Control 2	450008	NDM-1, OXA-48, VIM, IMP-7, CTX-M Group 1, CTX-M Group 2, CTX-M Group 9, TEM
ABR Positive Control 3	450009	ampC, ErmA, ErmB, FOX, KPC, SHV, TET(A), SUL1

PRIMER/PROBE MULTIPLEX CONFIGURATION

The panel is organized into **6 multiplexes (Plexes 1–6)**, each detecting 4 targets across FAM, HEX, Cy5, and ROX fluorescence channels. Select all four dyes for each labeled well.

Plex 1 — Target	**Channel**
ErmB	FAM
mecA	HEX
TET(A)	Cy5
bla-IMP / IMP-7	ROX
Plex 2 — Target	**Channel**
qnrA	FAM
qnrB	HEX
SHV	Cy5
qnrS	ROX
Plex 3 — Target	**Channel**
NDM-1	FAM
OXA-48, bla-OXA	HEX
KPC, bla-KPC	Cy5
VIM, bla-VIM	ROX

Plex 4 — Target	**Channel**
FOX	FAM
AmpC Gene	HEX
ACT	Cy5
ErmA	ROX
Plex 5 — Target	**Channel**
CTX-M Group 9	FAM
CTX-M Group 1	HEX
TEM	Cy5
CTX-M Group 2	ROX
Plex 6 — Target	**Channel**
vanA1_vanA2	FAM
SUL2	HEX
SUL1	Cy5
vanB	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 6 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: 500022) + **2.5 µL** nuclease-free water + **1 µL** 15X Primer/Probe Mix. Each of the 6 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 Master Mix (PN: 500022)	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

Vortex thoroughly before use.

Prepare each of the 6 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

*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

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SDS Requests

Safety Data Sheets available on request via phone or email

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