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Canine Enteric Pathogen qPCR Panel

Bandit Biosystems — Multiplex qPCR Primer/Probe & Controls Kit for Canine Enteric Pathogen Detection

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Description

The **Canine Enteric Pathogen qPCR Panel** is a qualitative, multiplex, nucleic acid, in vitro amplification assay used to detect pathogens typically associated with canine enteric pathogen infections or diarrhea. When paired with appropriate sampling and extraction methods, this panel is appropriate for use on specimens from veterinary sources.

Key Product Features

- Detects nucleic acid from 13 canine enteric pathogen targets
- Optimized for 96-well and 384-well formats at 15 µL reactions
- 4 multiplexed 15X Primer/Probe Mixes included
- Veterinary Enteric Pathogen Positive Controls included

Important Notices

- May not detect emerging novel mutated strains
- Amplification master mix kit must be purchased separately (PN: 500055)
- Use of alternate master mixes is not supported by Bandit Biosystems

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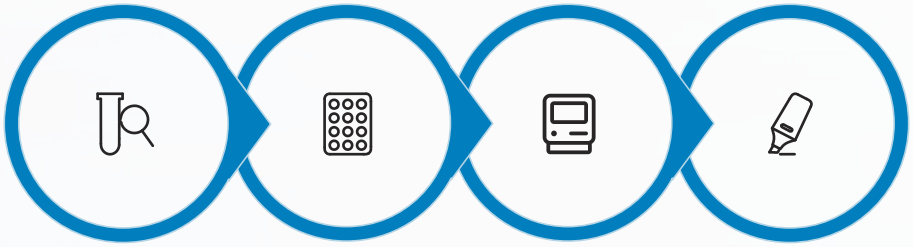
Target Organisms

The panel simultaneously detects nucleic acid from the following 13 canine enteric pathogen targets across bacteria, parasites, and viruses in a single multiplex qPCR workflow:

Target Pathogen	Target Pathogen
Clostridium difficile Toxin A/B	*Salmonella* spp
Clostridium perfringens enterotoxin (CPE)	*Cryptosporidium* spp
Clostridium perfringens alpha toxin (CPA)	*Campylobacter* (jejuni and coli)
Clostridium perfringens NetE / NetF	*Giardia* spp
Canine circovirus	Canine parvovirus
Canine distemper virus	*Lawsonia intracellularis*
Canine enteric coronavirus	

Test Principle

The Canine Enteric Pathogen qPCR Panel leverages **TaqMan-based multiplex RT-qPCR chemistry** to simultaneously detect multiple canine enteric pathogen targets in a single reaction. The **SPRY1 gene** is used as an internal sample integrity and extraction control — this novel multi-species control is positive for a variety of mammalian and avian species in addition to human DNA.



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

During amplification, the reverse transcriptase transcribes RNA into DNA for RNA targets. The 5' nuclease activity of Taq polymerase then 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 pathogen.

Step 1

Sample Extraction — isolate RNA and DNA using a validated extraction method

Step 2a

Master Mix Setup — combine 2X Master Mix, RT mix, nuclease-free water, and 15X Primer/Probe Mix as described in the 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 RT-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 Canine Enteric Pathogen qPCR Panel kit includes the primer/probe mixes and positive controls required for multiplex RT-qPCR. The amplification master mix must be purchased separately. The following kit configuration is available:

Kit 500053 — 100 Reactions

Plex 1 (PN: 400141), Plex 2 (PN: 400142), Plex 3 (PN: 400143), Plex 4 (PN: 400144) + Veterinary Enteric Pathogen Positive Control (PN: 450051) or individual controls (PN: 450055, PN: 450056, PN: 450057)

Component Details

Part Number	Description	Volume	Storage
400141–400144	15X Canine Enteric Pathogen qPCR Primer/Probe Mixes — (4) Multiplexes	(2) × 50 µL each	–20°C
450051 (or 450055, 450056, 450057)	Veterinary Enteric Pathogen Positive Control — mixed (or 3 individual control tubes)	(1) × 100 µL or (3) × 100 µL	–20°C

Recommended But Not Included

Part Number	Description	Volume	Storage
500055	Bandit Biosystems RT-qPCR Multiplex Amplification Kit 3000	(4) × 0.75 mL Master Mix + (4) × 0.05 mL RT mix + (1) × 1 mL Water	–20°C

Store all kit components at –20°C upon arrival. Only one master mix kit (PN: 500055) 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

SPRY1 primers and probes are included in the Primer/Probe Mix as an internal control, allowing control of the extraction procedure and detection of potential PCR inhibition. The SPRY1 assay detects DNA from most mammalian and avian species.

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. RNA controls serve as a reverse transcription control in addition to amplification control.

Controls Provided

Control	Part Number	Targets Included
Vet DNA Enteric A	450055	*Clostridium difficile* Toxin A/B, *Clostridium perfringens* enterotoxin (CPE), *Clostridium perfringens* alpha toxin (CPA), *Clostridium perfringens* NetE/NetF, *Cryptosporidium* spp, *Campylobacter* (jejuni and coli), *Salmonella* spp
Vet DNA Enteric B	450056	Canine circovirus, *Giardia* spp, Canine parvovirus, Lawsonia intracellularis, SPRY1 internal control
Vet Enteric RNA	450057	Canine distemper virus, Canine enteric coronavirus
Veterinary Enteric Pathogen Positive Control (mixed)	450051	All targets combined

PRIMER/PROBE MULTIPLEX CONFIGURATION

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

Plex 1 — Target	**Channel**
Clostridium difficile Toxin A/B	FAM
Clostridium perfringens enterotoxin (CPE)	HEX
Clostridium perfringens alpha toxin (CPA)	Cy5
Clostridium perfringens NetE / NetF	ROX
Plex 2 — Target	**Channel**
SPRY1 control	FAM
Canine circovirus	HEX
Canine distemper virus	Cy5
Canine enteric coronavirus	ROX

Plex 3 — Target	**Channel**
SPRY1 control	FAM
Salmonella spp	HEX
Cryptosporidium spp	Cy5
Campylobacter (jejuni and coli)	ROX
Plex 4 — Target	**Channel**
SPRY1 control	FAM
Giardia spp	HEX
Canine parvovirus	Cy5
Lawsonia intracellularis	ROX

HEX may be replaced by VIC or JOE if not present on the 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/RNA — Extract DNA/RNA from sample per a separate validated protocol as determined by the individual user.

02

Thaw Reagents — Completely thaw Master Mix, RT mix, nuclease-free water, and all 4 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 + **0.5 µL** RT mix + **2.0 µL** nuclease-free water + **1 µL** 15X Primer/Probe Mix. Each of the 4 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/RNA, 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 RT-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: 400098)	7.5 µL	1X
RT mix (PN: 400099)	0.5 µL	1X
Nuclease-Free Water	2.0 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 4 plex master mixes separately — do not combine.

Thermal Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
Reverse Transcription	1	1	50°C	15 minutes
RT Inactivation / 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). Cycling parameters reflect fast amplification on ABI instruments. Alternate instruments may require holding time adjustments.

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 < 40	+	Nucleic Acid Detected
Ct > 40	–	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 RT-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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