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# Feline Respiratory XL qPCR Panel

**Bandit Biosystems** — Multiplex qPCR Primer/Probe & Controls Kit for Feline Respiratory Pathogen Detection

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# Description

The **Feline Respiratory XL qPCR Panel** is a qualitative, multiplex, nucleic acid, in vitro amplification assay that detects pathogens typically associated with feline respiratory infections. 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 feline respiratory pathogen targets including bacteria and viruses
- Optimized for 96-well and 384-well formats at 15 µL reactions
- 2 multiplexed 15X Primer/Probe Mixes included
- Veterinary Respiratory Positive Controls included

### Important Notices

- May not detect emerging novel mutated strains
- Amplification master mix kit must be purchased separately (PN: 500046)
- Use of alternate master mixes is not supported by Bandit Biosystems

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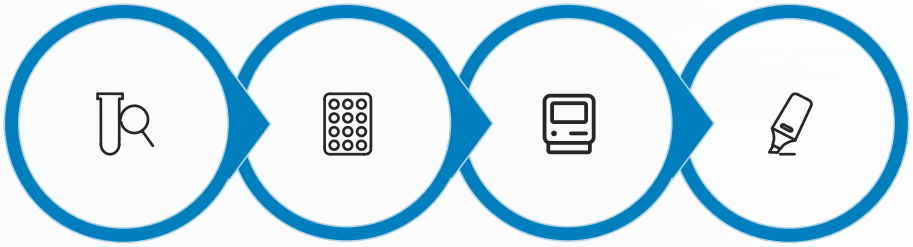
# Target Organisms

The panel simultaneously detects nucleic acid from the following feline respiratory pathogen targets across bacteria and viruses in a single multiplex qPCR workflow:

| Target Pathogen | Target Pathogen |
|---|---|
| Feline calicivirus | *Mycoplasma felis* |
| Influenza A (all strains) | Feline Herpesvirus 1 |
| *Chlamydophila felis* | SPRY internal control |
| *Bordetella bronchiseptica* | |

# Test Principle

The Feline Respiratory XL qPCR Panel leverages **TaqMan-based multiplex RT-qPCR chemistry** to simultaneously detect multiple feline respiratory 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 Feline Respiratory XL 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 500045 — 100 Reactions

Plex 1 (PN: 400103), Plex 2 (PN: 400104) + Feline Respiratory XL Positive Controls (PN: 450058, 450060 or 450044)

## Component Details

| Part Number | Description | Volume | Storage |
|---|---|---|---|
| 400103 | 15X Feline Respiratory XL qPCR Primer/Probe Mix 1 | (2) × 50 µL | –20°C |
| 400104 | 15X Feline Respiratory XL qPCR Primer/Probe Mix 2 | (2) × 50 µL | –20°C |
| 450058, 450060 or 450044 | Feline Respiratory XL Positive Control — individual control tubes or combined | (2) × 100 µL | –20°C |

## Recommended But Not Included

| Part Number | Description | Volume | Storage |
|---|---|---|---|
| 500046 | Bandit Biosystems RT-qPCR Multiplex Amplification Kit 1500 | (2) × 0.75 mL Master Mix + (2) × 50 µL RT mix + (1) × 1 mL Water | –20°C |

Store all kit components at –20°C upon arrival. 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 Resp RNA | 450058 | Feline calicivirus, Feline Herpesvirus 1, Influenza A (all strains) |
| Feline Respiratory DNA | 450060 | *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma felis*, SPRY internal control |
| Feline Respiratory Super Control Mixed | 450044 | All targets combined |

PRIMER/PROBE MULTIPLEX CONFIGURATION

The panel is organized into **2 multiplexes (Plexes 1–2)**, each detecting targets across FAM, HEX, Cy5, and ROX fluorescence channels. Select all four dyes for each labeled well.

| **Plex 1** — Target | **Channel** |
|---|---|
| SPRY internal control | FAM |
| Feline calicivirus | HEX |
| Influenza A (all strains) | Cy5 |
| (EMPTY) | ROX |

| **Plex 2** — Target | **Channel** |
|---|---|
| *Chlamydophila felis* | FAM |
| *Bordetella bronchiseptica* | HEX |
| *Mycoplasma felis* | Cy5 |
| Feline Herpesvirus 1 | 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 both 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 2 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 2 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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