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NOT FOR IN VITRO DIAGNOSTICS

NOT FDA APPROVED

Ringworm qPCR Panel

Bandit Biosystems — Multiplex qPCR Primer/Probe & Controls Kit for Ringworm Fungal Detection

Bandit
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Description

The **Ringworm qPCR Panel** is a qualitative, multiplex, nucleic acid, in vitro amplification assay that detects fungi species typically associated with ringworm 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 Microsporum spp and Trichophyton spp
- Optimized for 96-well and 384-well formats at 15 µL reactions
- 1 multiplexed 15X Primer/Probe Mix included
- Ringworm Positive Control included

Important Notices

- May not detect emerging novel mutated strains
- Amplification master mix kit must be purchased separately (PN: 500042)
- Use of alternate master mixes is not supported by Bandit Biosystems

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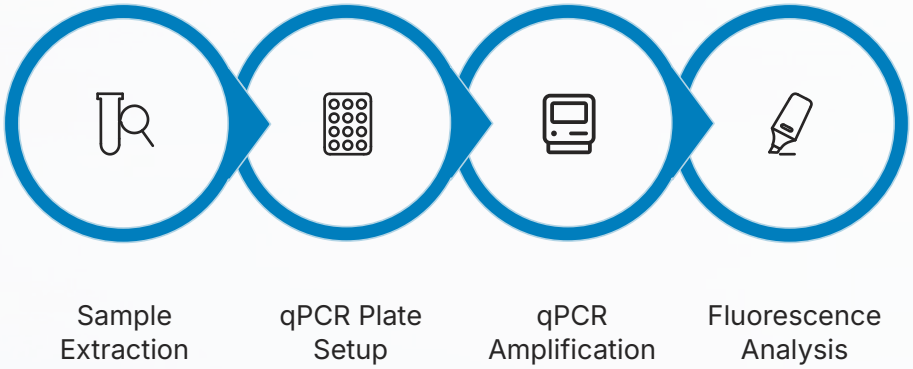
Target Organisms

The panel simultaneously detects nucleic acid from the following fungal targets associated with ringworm infections, along with an internal control for sample integrity verification:

Target	Notes
Microsporum spp	Detects multiple species including *M. canis*, *M. ferrugineum*, *M. audouinii*
Trichophyton spp	Detects multiple species including *T. mentagrophytes*, *T. tonsurans*, *T. equinum*
SPRY1 Internal Control	Detects DNA from most mammalian and avian species; confirms extraction integrity

Test Principle

The Ringworm qPCR Panel leverages **TaqMan-based multiplex qPCR chemistry** to simultaneously detect fungal 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.



The DNA Taq polymerase amplifies DNA fragments sharing homology with the primer/probe sets. During the amplification phase, the probe anneals to a specific target sequence. During extension, 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. Fluorescence intensity is monitored at each PCR cycle by the real-time PCR instrument.

Step 1

Sample Extraction — isolate DNA using a validated extraction method appropriate for the specimen type

Step 2a

Master Mix Setup — combine 2X Master 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 qPCR and analyze fluorescence data per channel

Required Instrumentation

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

Reagents, Kit Contents & Storage

The Ringworm qPCR Panel kit includes the primer/probe mix and positive control required for multiplex qPCR. The amplification master mix must be purchased separately. The following kit configuration is available:

Kit 500041 — 100 Reactions

Plex 1 (PN: 400097) + Ringworm Positive Control (PN: 450042)

Component Details

Part Number	Description	Volume	Storage
400097	15X Ringworm qPCR Primer/Probe Mix — Plex 1	(2) × 48 µL	−20°C
450042	Ringworm Positive Control	(1) × 100 µL	−20°C

Recommended But Not Included

Part Number	Description	Volume	Storage
500042	Bandit Biosystems qPCR Multiplex Amplification Kit 750	(1) × 0.75 mL Master Mix + (1) × 1 mL Water	−20°C

Store all kit components at −20°C upon arrival. The Bandit Biosystems qPCR Multiplex Amplification Kit 750 (PN: 500042) must be purchased separately. 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. Controls may also be diluted to a desired concentration and run directly.

Controls Provided

Control	Part Number	Targets Included
Ringworm Positive Control	450042	*Microsporum* spp, *Trichophyton* spp, SPRY1 Internal Control

PRIMER/PROBE MULTIPLEX CONFIGURATION

The panel is organized into **1 multiplex (Plex 1)**, detecting targets across FAM, HEX, and Cy5 fluorescence channels. Select all three dyes for each labeled well.

Plex 1 — Target	**Channel**
SPRY1 Internal Control	FAM
Microsporum spp	HEX
Trichophyton spp	Cy5

HEX may be replaced by VIC or JOE if not present on the instrument. Select all three 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 primer/probe mix 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 + **2.5 µL** nuclease-free water + **1 µL** 15X Primer/Probe Mix. 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

Vortex thoroughly before use. Select all three dyes (FAM, HEX, Cy5) for each labeled well.

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, and Cy5 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 < 40	+	Nucleic Acid Detected
Ct > 40	–	Nucleic Acid Not Detected

Pathogen (HEX/Cy5)	SPRY1 (FAM)	Result Interpretation
–	–	Invalid
–	+	Pathogen Nucleic Acid Not Detected
+/–	+	Pathogen Nucleic Acid 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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