Genient...

MYCOSCREEN18

Fungal Genomics

Genient

MYCOSCREE18

Fungus PCR Panel Kit

Instructions For Use (IFU)

Catalogue Numbers	GTPLMS18-096 · 96 Tests GTPLMS18-048 · 48 Tests
Storage	−15°C to −25°C · Protect from light

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1. Intended Use

The MYCOscreen18 Fungus PCR Panel Kit is a multiplexed qualitative Real-Time Polymerase Chain Reaction (qPCR) diagnostic test intended for the simultaneous in-vitro detection and species-level identification of eighteen (18) clinically relevant fungal pathogens from varied human specimens.

The kit is validated for use with the following amplification platforms: Bio-Rad CFX96 series, Gentier 96E Real-Time PCR System, and Applied Biosystems™ QuantStudio™ 5.

This kit is restricted to use by trained laboratory personnel in a clinical or research laboratory setting.

2. Product Description

Target DNA regions are amplified via real-time PCR using pathogen-specific primer and probe sets included in the kit. Each probe anneals to a specific target sequence flanked by forward and reverse primers. During PCR extension, the 5' nuclease activity of Taq DNA polymerase cleaves the probe, separating the fluorescent reporter dye from the quencher and generating a cumulative fluorescent signal proportional to the amount of target amplified.

Four fluorescent channels are utilised per reaction: FAM/Green, HEX/VIC/JOE/Yellow, ROX/Texas Red/Orange, and CY5/Red. An oligonucleotide set targeting human RNase P serves as the Internal Control (IC), monitoring sampling adequacy, nucleic acid extraction efficiency, and PCR inhibition.

A positive result is indicated by a real-time PCR sigmoidal growth curve and an associated Ct (Threshold Cycle) value of less than 38.

3. Kit Components

The MYCOscreen18 Fungus PCR Panel Kit consists of four main components:

Component	48 Test Pack	96 Test Pack
NP Master Mix (Lyophilised PCR Master Mix)	2 × 1,000 µL	4 × 1,000 µL
NP Oligo Mix 1–8 (Primer/Probe Sets for 18 targets)	8 × 125 µL	8 × 250 µL
NP Positive Control (PC)	1 × 400 µL	2 × 400 µL
NP Negative Template Control (NTC)	1 × 400 µL	2 × 400 µL

4. Additional Materials Required

The following materials are required but not supplied:

- Validated nucleic acid extraction consumables and instrumentation
- Real-Time PCR instrument with FAM™, HEX/VIC, ROX, and Cy5 detection channels
- Adjustable pipettes and filtered, nuclease-free pipette tips

- Appropriate personal protective equipment (laboratory coat, powder-free gloves, eye protection)
- Surface decontaminants: DNAZap™, DNA Away™, RNase Away™, or 10% sodium hypochlorite (1:10 dilution)
- Nuclease-free tubes, strips, or plates for dilutions and master mix preparation
- PCR-compatible plates or strips validated for the specific qPCR instrument in use
- Storage facilities capable of 4°C, −20°C, and −70°C (as applicable)
- Biosafety cabinet (recommended for PCR setup)
- Cold tube rack for microcentrifuge and PCR tubes

5. Storage Conditions

- Kits are shipped on dry ice (frozen).
- Store all components at −15°C to −25°C upon receipt.
- Do not exceed 3 freeze–thaw cycles; repeated cycling degrades sensitivity and fluorophore performance.
- Protect qPCR Master Mix vials from light at all times; prolonged exposure diminishes fluorophore activity.
- Keep reagents strictly segregated from clinical specimens to prevent cross-contamination.
- Do not use kit components beyond the labelled expiry date.
- If components show visible damage upon receipt, contact Genient Technologies immediately and do not use the kit.

6. Pre-Assay Considerations & Biosafety

6.1 Biosafety Requirements

- Wear appropriate PPE (gown, powder-free gloves, eye/face protection) when handling clinical specimens.
- All specimen processing must be conducted in a certified Class II Biological Safety Cabinet (BSC) under Biosafety Level 2 (BSL-2) or higher containment guidelines.
- Refer to: Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, CDC/NIH.
- Use of MYCOscreen18 is restricted to trained laboratory personnel only.

6.2 General Laboratory Precautions

- Perform equipment calibration checks before initiating any PCR run; do not use instruments without current maintenance records.
- Use validated qPCR plates/strips only; analytical performance specifications are valid solely with validated consumables.
- Decontaminate all work surfaces and equipment before and after use.
- Set up PCR reactions in a dedicated pre-PCR area, separated from post-PCR and extraction zones.
- Switch on the PCR system and load the appropriate kit protocol prior to reaction setup to minimise delay.
- Thaw all kit components on ice with gentle agitation and collect liquid at the tube bottom with a brief centrifuge spin before use.

7. Specimen Collection & Preparation

7.1 Appropriate Specimen Types

- Nail clippings from the affected nail
- Nail scrapings (subungual debris and nail plate material)
- Punch biopsy tissue from periungual or subungual regions
- Other clinical samples

7.2 Collection Guidelines

- Use sterile, single-use instruments for collection. Place specimens immediately in a sterile, sealed container.
- Store nail clippings and scrapings at 4°C; deliver to the laboratory within 24 hours. Avoid freezing unless specifically indicated.
- Tissue biopsy samples should be placed in sterile containers or appropriate transport media, stored at 4°C, and processed promptly.
- Label all containers clearly with patient ID, date, and sample type. Avoid extreme temperature exposure during transport.

7.3 DNA Extraction for PCR Assays

- The performance of this kit is critically dependent on the quality and purity of extracted DNA.
- Use only validated extraction kits/systems as listed in Section 1.
- Verify DNA purity (A260/A280 ratio ≥ 1.8) and quantify concentration prior to PCR setup.
- Extraction from nail and biopsy specimens may require prolonged lysis steps due to the keratin matrix; follow the extraction kit manufacturer's recommendations for Nail & Biopsies/fungal specimens.

8. Reaction Setup

8.1 Reaction Composition

Prepare the reaction mix for each well as follows (per-well volumes):

Reaction Mix Component	Volume per Well (1X)
NP Master Mix	5.0 µL
NP Oligo Mix (Mix 1–8, as applicable)	2.5 µL
DNA Isolate / Positive Control / NTC	2.5 µL
Total Reaction Volume	10.0 µL

Prepare the master mix (NP Master Mix + Oligo Mix) for the total number of patient samples plus 2 control reactions per run. Alternatively, distribute the NP Master Mix to all wells first, then add the Oligo Mix on top. Add the DNA isolate, Positive Control, or NTC last.

8.2 Target Organisms per Oligo Mix

Table below lists all 18 target organisms across 8 Oligo Mixes with their respective detection channels:

Oligo Mix	Target Organism	Detection Channel
Mix 1	*Epidermophyton floccosum*	FAM/Green
Mix 1	*Candida krusei*	HEX/VIC/JOE/Yellow
Mix 1	*Trichosporon asahii*	ROX/Texas Red/Orange
Mix 2	*Malassezia sympodialis*	HEX/VIC/JOE/Yellow
Mix 2	*Trichosporon mucoides*	CY5/Red
Mix 3	*Candida albicans*	FAM/Green
Mix 3	*Aspergillus flavus*	HEX/VIC/JOE/Yellow
Mix 4	*Aspergillus niger*	FAM/Green
Mix 4	*Candida glabrata*	HEX/VIC/JOE/Yellow
Mix 4	*Fusarium oxysporum*	CY5/Red
Mix 5	*Trichophyton spp.*	FAM/Green
Mix 5	*Candida auris*	CY5/Red
Mix 6	*Malassezia furfur*	FAM/Green
Mix 6	*Candida parapsilosis*	ROX/Texas Red/Orange
Mix 6	*Aspergillus terreus*	CY5/Red
Mix 7	*Aspergillus fumigatus*	ROX/Texas Red/Orange
Mix 7	*Human RNase P (Internal Control)*	CY5/Red
Mix 8	*Microsporum gypseum*	FAM/Green
Mix 8	*Microsporum canis / audouinii*	HEX/VIC/JOE/Yellow

9. PCR Thermal Protocol & Instrument Settings

9.1 Thermal Cycling Protocol

Step	Cycles	Temperature	Duration
Initial Denaturation	1	95°C	10 sec
Denaturation	40	95°C	5 sec
Annealing / Extension	40	55°C *	15 sec

* Enable data collection at the Annealing/Extension step for all four channels: FAM/Green, HEX/VIC/JOE/Yellow, ROX/Texas Red/Orange, and CY5/Red.

9.2 Instrument-Specific Threshold Settings

Apply the following settings before analysing results. Incorrect baseline or threshold configuration may produce spurious Ct values.

Instrument	Threshold Level	Additional Settings
Bio-Rad CFX96 Touch™ / CFX Opus 96™ / CFX384 Touch™ / CFX Opus 384™	500 RFU	—
QIAGEN Rotor-Gene Q 5plex	0.02 RFU	Dynamic Tube: Active; Slope Correct: Active; Outlier Removal: 0
Applied Biosystems™ QuantStudio™ 5, 7 & 12K	Auto	—
Roche LightCycler 480	Auto	—
Gentier 96E Real-Time PCR System	Per instrument manual	—

9.3 Run Validity — Control Acceptance Criteria

PC Target Channel	PC IC Channel	NC Target Channel	NC IC Channel / Decision
Positive (+)	**Positive (+)**	**Negative (−)**	Negative (−) → VALID RUN. Proceed to result interpretation.
Any negative	—	—	INVALID. Contact Genient Technical Support; renew reagents and repeat.
—	—	Any positive	INVALID. Check for contamination; repeat with attention to IFU precautions.

10. Interpretation of Results

MYCOscreen18 provides a qualitative result of Detected or Not Detected for each of the 18 target pathogens. Interpret sample results as follows:

Target Channel	Internal Control (RNase P)	Result	Interpretation
Positive (+) Ct < 38	Positive (+) Ct < 38	**POSITIVE**	Target DNA detected
Positive (+) Ct < 38	Negative (−) Ct ≥ 38 or N/A	**POSITIVE**	Target DNA detected (high copy number; IC outcompeted)
Negative (−) Ct ≥ 38 or N/A	Positive (+) Ct < 38	**NEGATIVE**	Target DNA not detected
Negative (−) Ct ≥ 38 or N/A	Negative (−) Ct ≥ 38 or N/A	**INVALID**	Repeat test by re-extracting sample. If invalid again, collect new specimen.

Note: A result is reportable only when the associated run has passed validity criteria (Section 9.3). A positive result indicates the presence of target DNA in the specimen; it does not quantify pathogen load. Clinical interpretation must be made in conjunction with clinical findings and other diagnostic information.

11. Performance Characteristics — Analytical Sensitivity (LoD)

The Limit of Detection (LoD) is defined as the lowest concentration at which ≥ 95% of replicates yield a positive result. Serial dilutions of ATCC/NCTC reference strains were tested; the tentative LoD was confirmed with 20 replicates. If initial detection was 100%, an additional 20 replicates were run at the next lower concentration until ≤ 95% was achieved. All tests were performed using CFX96 Touch™ (Bio-Rad) and validated extraction systems.

Analyte (Fungal Species)	Isolate ID / Source	LoD (copies/mL)	Detected / Total	Detection Rate
Microsporum gypseum	ATCC 14683	7.2×10^1	19/20	**95%**
Microsporum audouinii	ATCC 42558	8.9×10^1	20/20	**100%**
Microsporum canis	ATCC 36299	1.2×10^2	20/20	**100%**
Malassezia sympodialis	ATCC 42132	6.6×10^1	20/20	**100%**
Trichosporon asahii	ATCC 90039	6.5×10^1	20/20	**100%**
Epidermophyton floccosum	ATCC 9646	9.8×10^1	20/20	**100%**
Trichophyton soudanense	ATCC 24869	1.7×10^2	20/20	**100%**
Trichophyton terrestre	ATCC 24436	1.5×10^2	20/20	**100%**
Trichosporon mucoides	NCTC NCPF 8762	1.4×10^2	19/20	**95%**
Trichophyton tonsurans	ATCC 56186	1.6×10^2	20/20	**100%**
Trichophyton rubrum	Zeptometrix 0804478	8.7×10^1	20/20	**100%**
Trichophyton violaceum	ATCC 28944	8.8×10^1	20/20	**100%**
Trichophyton verrucosum	ATCC 28203	1.1×10^2	20/20	**100%**
Candida krusei	ATCC 2159	6.8×10^1	20/20	**100%**
Candida albicans	ATCC 10231	3.4×10^2	20/20	**100%**
Candida glabrata	ATCC 90030	4.4×10^1	20/20	**100%**
Candida parapsilosis	ATCC 22019	5.8×10^1	20/20	**100%**
Candida auris	ATCC MYA-5003	7.2×10^1	19/20	**95%**
Aspergillus niger	Zeptometrix 0801827	1.0×10^2	20/20	**100%**
Aspergillus flavus	Zeptometrix 0801598	9.8×10^1	20/20	**100%**
Aspergillus fumigatus	Zeptometrix 0801716	1.3×10^2	20/20	**100%**
Aspergillus terreus	Zeptometrix 0801601	8.7×10^1	19/20	**95%**
Malassezia furfur	ATCC 14521	1.2×10^2	20/20	**100%**
Fusarium oxysporum	ATCC MYA-1198	7.8×10^1	19/20	**95%**

Trichophyton mentagrophytes	ATCC 18748	9.8×10^1	20/20	**100%**
Trichophyton interdigitale	ATCC 9533	1.2×10^2	20/20	**100%**

12. Analytical Inclusivity

Analytical inclusivity was evaluated using a comprehensive panel of reference strains representing temporal, evolutionary, and geographic diversity for each target organism. Each strain was tested in triplicate at ≥ 1× the confirmed LoD. All 18 target organisms were detected at 1× LoD concentration across all three replicates. Where any replicate failed initially, retesting at 3× LoD was performed.

Result: 100% analytical inclusivity confirmed for all 18 reportable targets.

13. Analytical Specificity — Cross-Reactivity Evaluation

13.1 On-Panel Cross-Reactivity (Intra-Panel Interference)

All 18 on-panel organisms were tested in triplicate at ≥ 1.0×10^6 CFU/mL to evaluate potential intra-panel cross-reactivity. No cross-reactivity was observed.

Organism (Isolate / Source)	Cross-Reactivity
Microsporum gypseum (ATCC 14683)	**None Detected**
Microsporum audouinii (ATCC 42558)	**None Detected**
Microsporum canis (ATCC 36299)	**None Detected**
Malassezia sympodialis (ATCC 42132)	**None Detected**
Trichosporon asahii (ATCC 90039)	**None Detected**
Epidermophyton floccosum (ATCC 9646)	**None Detected**
Trichophyton soudanense (ATCC 24869)	**None Detected**
Trichophyton terrestre (ATCC 24436)	**None Detected**
Trichosporon mucoides (NCTC NCPF 8762)	**None Detected**
Trichophyton tonsurans (ATCC 56186)	**None Detected**
Trichophyton rubrum (Zeptometrix 0804478)	**None Detected**
Trichophyton violaceum (ATCC 28944)	**None Detected**
Trichophyton verrucosum (ATCC 28203)	**None Detected**
Candida krusei (ATCC 2159)	**None Detected**
Candida albicans (ATCC 10231)	**None Detected**
Candida glabrata (ATCC 90030)	**None Detected**
Candida parapsilosis (ATCC 22019)	**None Detected**
Candida auris (ATCC MYA-5003)	**None Detected**
Aspergillus niger (Zeptometrix 0801827)	**None Detected**
Aspergillus flavus (Zeptometrix 0801598)	**None Detected**
Aspergillus fumigatus (Zeptometrix 0801716)	**None Detected**

Aspergillus terreus (Zeptometrix 0801601)	**None Detected**
Malassezia furfur (ATCC 14521)	**None Detected**
Fusarium oxysporum (ATCC MYA-1198)	**None Detected**
Trichophyton mentagrophytes (ATCC 18748)	**None Detected**
Trichophyton interdigitale (ATCC 9533)	**None Detected**

13.2 Off-Panel Specificity (Near-Neighbours & Common Flora)

Off-panel organisms — including near-neighbour fungi, common flora, and clinically similar pathogens — were tested in triplicate at ≥ 1.0×10^6 CFU/mL. In silico whole-genome sequence analysis was performed for organisms unavailable for wet testing. No cross-reactivity was observed.

Organism (Isolate / Source)	Cross-Reactivity
Scopulariopsis brevicaulis (ATCC 36840)	**None Detected**
Aspergillus versicolor (Zeptometrix 0801822)	**None Detected**
Pseudomonas aeruginosa (Zeptometrix 0801908)	**None Detected**
Candida lusitaniae (Zeptometrix 0801603)	**None Detected**
Candida dubliniensis (Zeptometrix 0801915)	**None Detected**
Bartonella quintana (Zeptometrix 0804360)	**None Detected**
Bartonella henselae (Zeptometrix 0804359)	**None Detected**
Aspergillus clavatus (ATCC 20062)	**None Detected**
Aspergillus nidulans (ATCC 10074)	**None Detected**
Candida guilliermondi (Zeptometrix 0801602)	**None Detected**
Aspergillus oryzae (ATCC 1011)	**None Detected**
Candida humilis (ATCC 22992)	**None Detected**
Fusarium solani (Zeptometrix 0801806)	**None Detected**
Scopulariopsis asperula (ATCC 58360)	**None Detected**
Paecilomyces variotii (ATCC 26820)	**None Detected**
Acremonium acutatum (ATCC 32209)	**None Detected**
Microsporum gallinae (ATCC 22242)	**None Detected**
Malassezia dermatis (ATCC MYA-4955)	**None Detected**

14. Troubleshooting

Problem	Probable Cause	Recommended Action
Target-specific and/or IC signals detected in the Negative Control well.	Environmental contamination; contamination of extraction and/or qPCR reagents; well-to-well cross-contamination.	Repeat qPCR with fresh reagents. Decontaminate all surfaces with 10% sodium hypochlorite or 70% ethanol. Use filtered tips; change between samples. Set up reactions in a designated pre-PCR area. Ignore Ct if

		amplification curve resembles background noise.
No IC signal detected, but target-specific signal present in sample wells.	High copy number of target nucleic acid causes preferential amplification of target over IC.	No corrective action required. Result is considered POSITIVE.
Positive Control did not meet acceptance criteria. Assay invalid.	Positive Control stored under incorrect conditions, or kit past expiry date.	Check storage conditions and expiry date on kit label. Replace Positive Control. Use a new kit if necessary.
High Ct values observed for repeated samples.	Kit components not mixed thoroughly after thawing; degraded nucleic acids.	Thaw components with mild agitation. Ensure kit is stored correctly and not subjected to more than 3 freeze–thaw cycles.
Target-specific and/or IC signal detected after Cycle 38 in Positive Control.	Incorrect qPCR setup; reagents may be compromised due to improper storage or excessive freeze–thaw cycles.	Replace the Positive Control. If the problem persists, contact Genient Technical Support.
No target-specific signal and no IC signal detected in sample wells.	Sampling, extraction, or PCR inhibition problem.	Dilute the nucleic acid isolate 1:10 and repeat qPCR. If IC remains negative in diluted sample, request a new specimen and repeat NA extraction. If problem persists, contact Genient Technical Support.

15. Symbols Reference

Symbol	Meaning	Symbol	Meaning
CE IVD	In-vitro diagnostic device	REF	Catalogue / reference number
LOT	Batch code	Σ	Contains sufficient for <n> tests
CONTROL −	Negative control	CONTROL +	Positive control
⚠	Caution — consult IFU	i	Consult instructions for use
NON STERILE	Non-sterile product	↕↕	Keep upright
🌡	Temperature limit	☀ (crossed)	Keep away from sunlight
☂	Keep dry	☢ (crossed)	Protect from heat and radioactive sources
(hourglass)	Use-by date	∅	Do not use if package is damaged
▲ (manufacturer)	Manufacturer details	—	—

16. Contact & Legal

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