



# Fungal Microbiota Panel

## 96 Well

### Product Specification Sheet

RUO Product\*

### Product Description:

The Fungal Microbiota Panel is an in-vitro multiplex real-time polymerase chain reaction (RT-PCR) assay for the qualitative identification of nucleic acids from fungi and bacteria. This method is highly accurate, analytically sensitive, and is used to identify organisms by amplifying and detecting genetic material of pathogens in samples. The panel will aid in the research of causative agents of fungal infections and their prevalence.

The target organisms included in the panel are as follows: **Alternaria spp., Aspergillus spp., Epidermophyton floccosum, Scytalidium dimidiatum, Fusarium spp., Curvularia spp., Candida albicans, Candida glabrata, Candida krusei, Candida parapsilosis, Candida tropicalis, Cryptococcus spp., Trichosporon spp., Malessezia spp., Trichophyton anthropilic spp.**(targets include: *T. rubrum*, *T. violaceum*, *T. soudanense*, & other species) **Trichophyton zoophilic spp.** (targets include: *T. mentagrophytes*, *T. interdigitale*, *T. tonsurans*, *T. verrucosum*, *T. simii*, *T. benhamiae*, *T. equinum*, & other species), **Meyerozyma guilliermondii, Microscporum canis, Sarocladium strictum.** The panel also includes bacterial add on *Pseudomonas aeruginosa* and the antibiotic resistance marker, *mecA*.

Product Information	
Urinary Tract Microbiota Plates (96 well Plate)	
Part Number	P-FUN096-101-A P-FUN096-002-A P-FUN096-003-A P-FUN096-004-A
Number of Panels	6
Storage Temperature	-25°C to -15°C

Product Specifications	
QC Test	qPCR Cycle Threshold Percent CV
Specification	≤ 2.5

QC Results	
Positive	meets specification
Negative	meets specification
Targets	meets specification

### ▶ Limitations of Use

Molecular Designs products are for Research Use Only and intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

### ▶ \* Limitations of Use

For Research Use Only. Not for use in diagnostic procedures.

### ▶ Product Guarantee

This kit is proven in PCR and generates reliable, repeatable and high-performance results. Please contact Molecular Designs for technical assistance. If not completely satisfied, our team will help you identify and address the issue and replace the assays as needed.

*Jaspreet Seth*

**Jaspreet Seth**

Vice President, Quality  
Molecular Designs, LLC

# Usage Information



## ▶ Reagent Storage and Use Guidelines

1. Store all reagents at -25°C to -15°C.
2. Do not freeze-thaw plates more than 3 times.

## ▶ The Following is Included in the Kit:

1. 96-well PCR plate pre-loaded with the Fungal Microbiota assays and positive and negative controls.

## ▶ The Following is Supplied by the User: Materials

1. Extracted Sample(s)
2. qPCR optical film
3. Sealer for optical film

## ▶ Equipment

1. Manual defrost -20°C freezer
2. Laminar Flow or PCR Dead Air Box for general plate setup. Do not use Laminar Flow for infectious samples
3. Pipettes and appropriate filtered pipette tips
4. Plate Vortex [recommend Vortex Genie 2 (Model G560) with the 3-inch platform and rubber cover]
5. Plate centrifuge
6. Lab utility knife

## ▶ Instrumentation

1. CFX96 Touch Real-Time PCR Detection System (or equivalent)

## ▶ General Guidelines and Safety Precautions

1. As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- a) Do not pipette by mouth.
- b) Do not eat, drink, or smoke in designated work areas.
- c) Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- d) Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- e) Thoroughly clean and disinfect all laboratory work surfaces.

**NOTE:** Do not use sodium hypochlorite solution (bleach) to clean up a spill or to disinfect a plate before disposal as it can react with the common extraction reagents and generate toxic byproducts. If spills occur, follow internal procedures to immediately clean and decontaminate the surface of instrument.

2. A laminar flow or PCR Dead Air Box is recommended to reduce contamination probability.
3. The use of filtered, sterile and nuclease-free pipette tips is recommended.
4. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.



# Usage Information



## ▶ Reaction Plate Setup

1. Remove a reaction plate from the -20°C manual defrost freezer.
2. For the Breakaway plates (P-FUN096-101-A and P-FUN096-002-A), determine the number of panels that will be used from the reaction plate, score the foil seal on the PCR break-away plates using the lab utility knife and tear the plate along the perforated edge between samples wells to obtain the number of panels needed. Ensure the excess panels on the plate are properly labelled and sealed and promptly place the remainder back in the -20°C freezer.
3. Use the plate within 1 hour of thawing, keep sealed and store refrigerated at 4°C if not using immediately.
4. Spin down the plate for 30 seconds in a plate centrifuge.
5. Carefully remove the foil seal from the plate.
6. Add 4.0 µL of the sample being tested to each of the target wells.
7. Do not add any additional liquid to the Positive Control and Negative Control wells. All components have been added to these wells.
8. Seal the PCR plate using optical qPCR film. Note: If using a partial plate, remove the excess optical seal using the utility knife and ensure the plate is sealed.
9. Vortex the plate, at least 5 seconds per plate quadrant.
10. Spin down the plate in a plate centrifuge.

## ▶ Procedural Notes

1. Do not reuse consumables. They are for one-time use only.
2. Always use caution when transferring specimens from a primary collection tube to a secondary tube.
3. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens.
4. Always use a new pipette tip for each specimen.
5. For testing of previously frozen sample, ensure samples are equilibrated to room temperature and well mixed prior to use.

## ▶ Plate Layout (96 well plate, 4 panels, each panel)

Alternaria spp. / Aspergillus spp. (CFO)	Scytalidium dimidiatum (CFO)
Epidermophyton floccosum (CFR)	P. aeruginosa
Fusarium spp. / Curvularia spp. (CFR)	mecA
C. albicans / C. glabrata (CFO) / C. krusei (CFR)	Endogenous Control
C. tropicalis / C. parapsilosis (CFR)	
Cryptococcus spp. / Trichosporon spp. (CFR)	
Trichophyton anthropophilic spp. / Malassezia spp. (CFO) / Trichophyton zoophilic spp. (CFR)	Positive Control
Meyerozyma guilliermondii / Microsporum canis (CFO) / Sarocladium strictum (CFR)	Negative Control

*If not noted, the fluorophore is FAM and the second channel is noted in parentheses signifying a secondary target in the primer and probe mixture which will be detected on the qPCR instrument. CFO is equivalent to CAL Fluor Orange 560 and CFR is equivalent to CAL Fluor Red 610.*



# Usage Information



## ▶ Real-Time PCR Detection System qPCR Run Setup

1. Open the specified run template and fill in the sample name fields with unique sample IDs corresponding to the samples being processed.
2. **NOTE:** This step can also be done prior to reaction plate setup if sample IDs have already been specified.
3. Place the reaction plate into the instrument in the appropriate orientation (A1 in the upper left corner), close the instrument lid and initiate the run.

## ▶ Thermocycling Protocol

1. Reverse Transcription
  - a) 5 minutes at 50°C
2. Denaturation
  - a) 3 minutes at 95°C
3. Annealing and Extension
  - a) 40 cycles consisting of:
    - a) 5 seconds at 95°C
    - b) 30 seconds at 60°C, with fluorescence acquisition during this step

## ▶ Amplification Interpretation and Troubleshooting

1. The laboratory should establish cycle threshold (CT) cutoffs as appropriate for their sample workflow and procedures. It is recommended that CT cutoffs are determined during the validation of the test.
2. The laboratory should evaluate the curve shape when considering whether a sample with a given CT should be considered positive:
  - a) Plate sealing issues can lead to jagged curve shapes or rising/decreasing baselines that lead to inaccurate data (erroneous CT value).
  - b) Inappropriate mixing or centrifuging can lead to inaccurate data.
3. If user suspects contamination, it is recommended to clean and disinfect the laboratory area and re-test to ensure proper results.
4. Any failure of the positive or negative control should require a repeat run. If the control failure continues, it is recommended to have the qPCR instrument and the sample extraction workflow evaluated to ensure they are functioning properly.

