PRIME DISCOVERIES

315 Madison Avenue, Suite 3081
New York, NY, US 10017
Phone: 646-760-9063
E: contact@primediscoveries.com
W: www.primediscoveries.com

PRIME AurisDetect™ RAPID ASSAY

INSTRUCTIONS FOR USE

Prime AurisDetect™ qPCR kit for the detection of *Candida auris*

Candida auris is an emerging fungus that presents a serious global health threat. It is often multidrug-resistant, meaning that it is resistant to multiple antifungal drugs commonly used to treat Candida infections. Some strains are resistant to all three available classes of antifungals. It is difficult to identify with standard laboratory methods, and can be misidentified in labs without specific technology. Misidentification may lead to inappropriate management. It has caused outbreaks in healthcare settings. For this reason, it is important to quickly identify *C. auris* so that healthcare facilities can take special precautions to stop its spread.

INSTRUCTIONS FOR USE

Prime Discoveries AurisDetect™ is PCR-based and requires Mawi iSwab-EL Extraction-less Buffer

1. PURPOSE

The purpose of this procedure is to describe the process for identification of *Candida auris* using the Applied Biosystems 7500 Fast Real-time PCR platform or QuantStudio instruments.

2. PROTOCOL

The protocol is based on the modification of using Mawi's iSwab-EL Extractionless buffer. This buffer allows skipping the DNA extraction step. If no Mawi's iSwab-EL Extraction-less buffer is used please perform gDNA extraction.

For collecting the specimens in Mawi Buffer, place the swab into the Mawi's iSwab-EL Extraction-less buffer. Please contact Mawi DNA Technologies for any question related to collection and samples storage.

3. EQUIPMENT & MATERIALS

- Biological Safety Cabinet Certified Class II A2
- PCR enclosure
- Applied Biosystems 7500 Fast Real-time PCR instrument with SDS Software version 1.4 or QuantStudio version 3, 5, or 6/7 Flex Systems
- Calibrated pipets (P2/10, P20, P200, P1000)
- Aerosol barrier (filter) tips
- 96 well cooling rack
- RNase/DNase-free 1.5mL microcentrifuge tubes
- MicroAmp Fast Optical 96-Well Reaction Plate, 0.1mL
- MicroAmp Adhesive optical film
- Molecular Grade RNase/DNase free water
- *Candida auris* primers and probes
- RNAse specific primers and probes
- Prime AurisDetect™ Enzyme mix
- DNA from Specimens
- 10% bleach solution, or other C. *auris*-approved cleaning agent
- 70% Ethanol
- *Candida auris* Control (ATCC MYA-5001) provided in Mawi Buffer by Prime Discoveries
- Human Genomic DNA (negative control for C.*auris*)

4. SAFETY PRECAUTIONS

- Clinical *Candida auris* specimens (Swabs/Sponge) or isolates have the potential to transmit infectious diseases. Wear protective gloves, a lab coat, and eyewear when handling samples.
- All BSL-2 practices, safety equipment, and facility design must follow the requirements described in the BMBL and the Biosafety Manual.

5. SPECIMEN AND REAGENT STORAGE

- Specimen tubes received for testing must be intact, labeled with two unique identifiers (e.g. patient ID and specimen ID), and must be accompanied by a submission form.
- All patient swabs are collected using the appropriate Mawi's Extraction-less buffer/ tubes (contact the Manufacturer for instructions and cat#).
- Specimens should be stored at 4°-25°C, and shipped with a cold pack to the laboratory for processing within 96 hours of collection.

Specimen Rejection Criteria

- Specimens received >4 days after collection.
- Damaged or visible leakage of transport tubes.
- A specimen without submission forms.
- Specimen without identifiers.
- Individual institutions submitting swabs are responsible for compliance with local human subjects/Institutional Review Board (IRB) regulations or applying for the IRB exemption for public health outbreak surveillance and emergency response.

Reagent Handling and Storage

- Store all primers and probes at 2-8°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on ice or cold block at all times during preparation and use.
- Control DNA must be thawed and kept on ice at all times during preparation and use.
- Clean and decontaminate all work surfaces, BSC, pipettes, and other equipment with a proper C.*auris*-approved decontamination solution such as 10% bleach solution before and after use.
- Use separate and dedicated equipment (e.g., BSC, pipettes) and supplies (e.g., microcentrifuge tubes, pipette tips) for master mix preparations, setup of Optical 96-well reaction plate, and addition of DNA template in the wells and decontaminate with UV light and/or ELIMINase to prevent DNA contamination after work is completed.
- Reagents, master mix, and DNA should be maintained on the cold block during preparation and/or use to ensure stability.

6. QUALITY CONTROL

PCR Controls

- A Positive and Negative PCR control should be included with each PCR reaction.

Prepare a C.*auris* positive PCR control

- Use the positive control provided with the Prime Discoveries Kit. It contains *Candida auris* in Mawi's Buffer.
- There should be a C.*auris* positive result for the PCR positive control.
- If there is a C.*auris* negative result, invalidate patient specimen results and rerun the PCR.

Prepare a C.*auris* negative PCR control

- Prepare a PCR negative control by adding Human genomic DNA into the reaction.
- Negative Control should return C.*auris* negative and RNAse positive.
- If there is a C.*auris* positive result for the Human Genomic DNA, invalidate patient specimen results and retest.

Prepare a No Template Control (NTC)

- NTCs consist of PCR-grade water in place of input DNA.
- NTC should be included in each run NTCs.
- NTCs should return C.*auris* negative and RNAse negative.
- If the result is C.*auris* positive or RNAse positive, invalidate patient specimen results and rerun the PCR, starting post-extraction.

Replication

- All samples should be run in triplicate. If there are discordant values between the triplicates then the majority two values take precedence.

7. PREPARE MASTER MIX REACTION

- Prepare the Master Mix reaction as illustrated below:
- The following protocol is specific for the Prime AurisDetect™ Enzyme mix

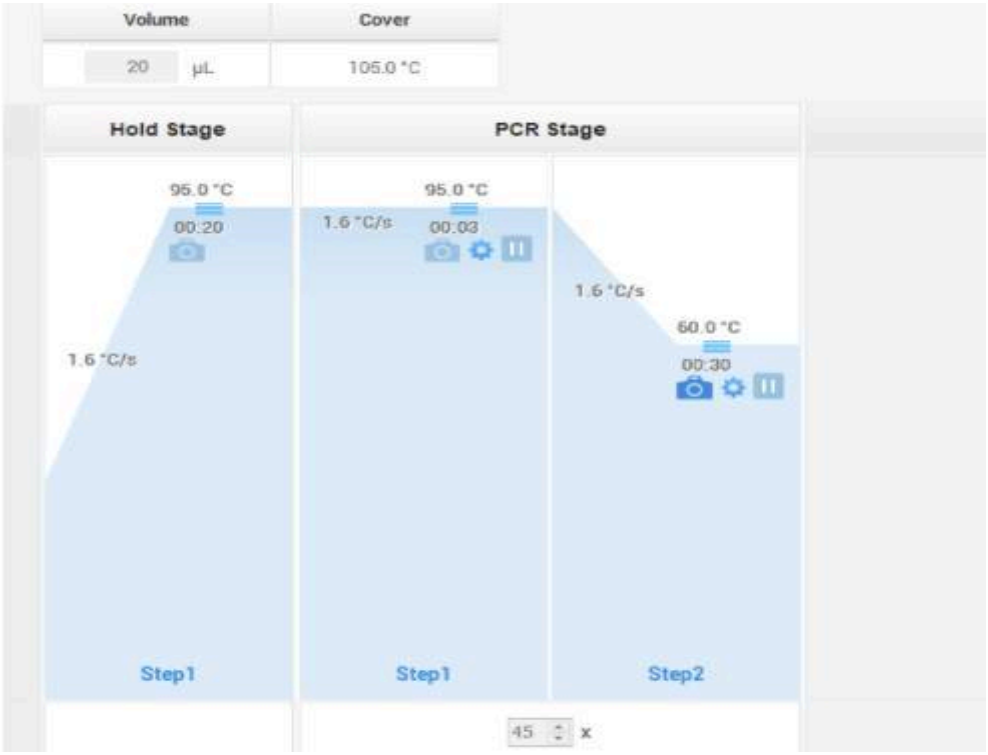
Reagent (stock concentration)	Volume/Rxn (microliters)
Prime AurisDetect™ Enzyme mix	10.0
C.*auris* Forward Primer (CAURF) 10uM	1.0
C.*auris* Reverse Primer (CAURF) 10uM	1.0
C.*auris* probe CAURP 2.5 uM	0.8
RNAse P Forward Primer 10uM	1.0
RNAse P Reverse Primer 10uM	1.0
RNAse Probe BICP 2.5 um	0.8
Water	0.4
Total Volume	**16.0**

- Add Master Mix (16 microliters) and then add 4 microliters of template DNA (if using Mawi iSwab-EL buffer add 4 microliters of sample directly into the reaction). The final volume should be 20 microliters per reaction.
- *Candida auris* should be set to the FAM channel RNase P should be set to the Cy5 Channel.

8. SET UP PCR REACTION

- Modify the thermal cycling conditions as follows:
- In Stage 1, Set to 20 Sec at 95°C; 1 Rep
- In Stage 2, Step 1 set to 03 Sec at 95°
- In Stage 2, Step 2 set to 30 Sec at 60°C
- In Stage 2, Reps should be changed to 45
- Under Setting, bottom left box, set volume to 20 µL
- Stage 2 Step 2 should be highlighted to indicate data collection

9. PCR SETUP EXAMPLE



10. DATA ANALYSIS

- After the run has been completed perform data analysis.
- Cutoff values must be determined by each laboratory during the validation phase. Cutoff values can vary between different extraction methods and by sample type. Determine the specific cutoff value for each extraction method and sample type. The cutoff value established for this assay is Ct_{cutoff}
- A sample with a Ct value ≤ Ct_{cutoff} is positive for C.*auris*