URINARY TRACT INFECTION (UTI)

Contents	Storage
Primers and Probes are spotted in the plate (refer to Tables 3-5 for layout). This is a collection of multiplex assays that detect microbes and/or antimicrobial resistance, as well as an internal control in the same reaction.	Pre-Spotted Plates are shipped on dry ice. Upon receipt, store as follows: –15 to –30°C in a constant temperature (non–frost free) freezer until the indicated expiration date Notes: • Avoid repeated freezing and thawing. • Avoid prolonged exposure of material to light. • Product will arrive solid and should be thawed at 4°C or on ice. Product should remain frozen in the –20°C freezer.

REAGENT DESCRIPTION

This urinary tract infection (UTI) pathogen polymerase chain reaction (PCR) pre-spotted plate is intended for qualitative, or semi-quantitative, detection of urinary tract pathogens.

MATERIALS AND EQUIPMENT TO BE SUPPLIED BY THE USER

The following list includes materials that are required for use but not are not included.

- Real-time PCR equipment
- Dye calibration plates (if needed)
- Minicentrifuge
- PCR Plate centrifuge
- Vortex Mixer
- Extraction equipment and associated reagents
- Biological safety cabinet or hood
- Pipettes (single and/or multi-channel) (10 µL, 200 µL, 1000 µL)
- Filtered pipette tips
- PCR plate sealing film
- PCR film applicator
- PCR Clean 1.5- or 2 mL tubes
- Biohazard waste containers
- Specimen racks
- Nuclease-free water
- Cold block
- Disposable gloves

EXPERIMENTAL

ADD MASTERMIX

1. Add MasterMix to the wells of the qPCR plate that contain Primer Mix. Note: Follow guidelines based on MasterMix Product Insert

ADD DNA/RNA TEMPLATE

2. Add direct specimen, extracted RNA template, or controls, to the wells of the qPCR plate that contain Primer Mix. Note: At this point, the total volume should be 10 µL.
3. Seal the qPCR plate with optically transparent film.
4. Agitate by mixing or briefly vortexing, then centrifuge briefly to remove air bubbles and collect the reaction at the bottom of the wells.

SET UP THE PCR CYCLING PROGRAM

Program the appropriate PCR cycling protocol on your real-time PCR instrument (refer to Table 2).

TABLE 2. PCR CYCLING PROTOCOL.

Standard RT-qPCR Reaction using purified RNA				
Step	Cycles	Temperature (°C)	Standard Cycling (min:sec)	Fast Cycling (min:sec)*
Reverse Transcription	1	50	15:00	15:00
Polymerase activation	1	95	3:00	0:30
Amplification:				
Denaturation	35-45	95	0:15	0:05
Annealing/Extension†		60	1:00	0:45

* Fast cycling parameters may (in some cases) reduce sensitivity and/or signal.
† This is a general starting point. The ideal annealing/extension temperature or time may need to be empirically determined