

GENERAL INFORMATION

Contents	Storage
Primers and Probes are spotted in the plate. 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 –25°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. • The product will arrive solid and should be thawed at 4°C or on ice. Products should remain frozen in the –20°C freezer.

REAGENT DESCRIPTION

This 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 are not included.

Real-time PCR equipment	PCR plate (96 or 384 well, clear or white)
Dye calibration plates (if needed)	PCR plate sealing film
Minicentrifuge	PCR film applicator
PCR Plate centrifuge	PCR Clean 1.5- or 2 mL tubes
Vortex Mixer	Biohazard waste containers
Extraction equipment and associated reagents	Specimen racks
Biological safety cabinet or hood	Nuclease-free water
Pipettes (single and/or multi-channel) (10 µL, 200 µL, 1000 µL)	Cold block
Filtered pipette tips	Disposable gloves

EXPERIMENTAL

PREPARE REACTION MIX

1. Determine the number of reactions needed for your experiment, including replicates, controls (no template control; positive control), and 1–3 additional reactions to adjust for pipetting differences.
2. Combine all components, except for the DNA/RNA template (Table 1), and thoroughly mix.

Notes:

- DNA/RNA template will be added to the Reaction Mix in the qPCR plate in the Add DNA/RNA template section.
- The volumes provided in Table 1 are per reaction; calculate the final component volumes by multiplying each volume by the total number of reactions.
- Pre-spotted plates already have the Primer Mix aliquoted into them

TABLE 1. REACTION MIXES.

Standard RT-qPCR Reaction using purified RNA			
Component		Volume per 384 well reaction	Volume per 96 well reaction
RM Mixture	PrimeTime One-Step 4X		
	Broad-Range Master Mix (4X)	2.5 µL	5 µL
	Primer Mix	5 µL	10 µL
Sample	RNA/DNA Template (do not add yet)	2.5 µL	5 µL

Direct Amplification RT-qPCR Reaction			
Component		Volume per 384 well reaction	Volume per 96 well reaction
RM Mixture	PrimeTime One-Step 4X		
	Broad-Range Master Mix (4X)	2.5 µL	5 µL
	Primer Mix	5 µL	10 µL
Sample	RNA/DNA Template (do not add yet)	1.5 µL	3 µL
Direct Amplification	Direct Amplification Enhancer*	1 µL	2 µL

*The Enhancer is generally used for crude specimens. For NP samples, store directly in VTM/UTM. For liquid samples (saliva, other) store at a 1:1 ratio in VTM/UTM. As a guideline, testing with a broad survey of VTM/UTM formulations indicates that 1 µL of Direct Amplification Enhancer per 20 µL is appropriate for saliva samples stored in VTM.

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