Apostle MiniMax™ High Efficiency Urinary Tract Microbiota DNA Isolation Automation Protocol on Apostle MagTouch 2000 Automation Platform – 400 µL Urine (concentrated from 10mL) Extraction

Version 1.4

Product description

The Apostle MiniMax™ High Efficiency Bacterial DNA Isolation Kit is designed for isolation of bacterial DNA from urine samples. The kit uses proprietary Apostle MiniMax™ technology, offers highly efficient, reproducible recovery of high-quality bacterial DNA with high yield. The isolated DNA samples are suitable for a broad range of subsequent applications, including sequencing, PCR, etc. The protocol is designed for 96-well plate automated on Apostle MagTouch 2000 Automation Platform. Especially for samples with extreme low dilute bacteria concentration, centrifugal and concentration pretreatment could increase the total bacteria DNA product.

Required materials

- Apostle MagTouch 2000
- Apostle 96-well plates and 96-tip comb
- Adjustable single and multi-channel micropipettes (1 mL, 200 µL, 20 µL) and tips
- DNase/RNase-free tubes (1.5 mL and 15 mL)
- Liquid reservoirs
- Vortex or shaker
- Ethanol, 200 proof
- DNase/RNase free water
- Table top centrifuge
- Heater (for sample lysis. *Not needed if lysis on automation platform*)

Procedure

A. Urine sample pretreatment

1. Gently invert the urine samples to ensure thorough mixing.

2. For each urine sample, transfer 10 mL urine into a 15 mL tube.

3. Centrifuge the tubes at 2,250 × g for 15 minutes to concentrate the samples.

4. Carefully remove 9.56 mL of supernatant with pipette (there may not be an obvious pellet and be careful not to disturb the pellet)

5. Vortex to fully resuspend the pellet (Optional: if needed, transfer the 400 µL samples to 1.5 mL tubes)

B. Sample lysis (2 options)

1. Lysis on heater (method I)

1.1 Add components to the sample tube in the order indicated below.

400 µL concentrated urine:

Reagents	Volume
Urine (pre-existing)	400 µL
Protease K	16 µL
Mix both well with pipette or vortex	
Sample Lysis Buffer	40 µL

Caution: avoid mixing proteinase K with Sample Lysis Buffer before Urine.

1.2 Mix the solution well by vortexing briefly and incubate the mixture at 60°C for 20 minutes.

1.3 At the end of the incubation, cool the tubes containing the urine to room temperature.

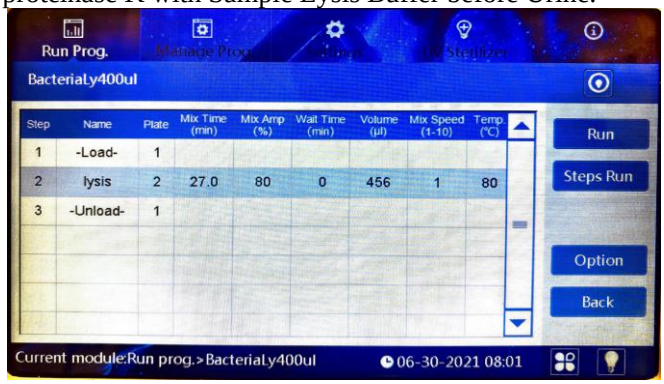
2. Lysis on automation platform (method II)

2.1 Add components to the 96-well plate for lysis in the order indicated below.

400 µL concentrated urine:

Reagents	Volume
Protease K	16 µL
Urine	400 µL
Sample Lysis Buffer	40 µL

Caution: avoid mixing proteinase K with Sample Lysis Buffer before Urine.



2.2 Select the <BacteriaLy400ul> program. Open the cabin door, place the lysis plate into the Apostle MagTouch 2000 as indicated on the instrument display (Plate Position 2).

2.2 After placing the lysis plate, press the <Run> icon on the interface to start the program.

2.3 When the lysis program completes, cool the plate containing the urine to room temperature for next steps.

C. Bacterial DNA extraction

1. Set up the 96 well plates according to the table below outside the instrument:

400 µL concentrated urine:

Plate Position	Plate Type	Name	Content	Reagent volume for each well
1	96 well plate with comb	-Load-	Comb	-
3	96 well plate	bind	lysed sample from step A	456 µL
			Bacterial DNA Lysis/Binding Solution	500 µL
			Magnetic Nanoparticles	6 µL
4	96 well plate	wash1	Bacterial DNA Wash Solution	750 µL
5	96 well plate	wash2a	prepared secondary wash buffer (20% Apostle MiniMax™ Bacterial DNA 2nd Wash Solution, with 80% Ethanol)	750 µL
6	96 well plate	wash2b	prepared secondary wash buffer (20% Apostle MiniMax™ Bacterial DNA 2nd Wash Solution, with 80% Ethanol)	750 µL
7	96 well plate	elu	Bacterial DNA Elution Solution	60 µL

2. Automated bacterial DNA extraction on Apostle MagTouch 2000 Automation Platform:

2.1 Select the <BacteriaEx400ul> program. Open the cabin door, place the plates into the Apostle MagTouch 2000 as indicated on the instrument display. Press the position button to turn the rotary table and place all the plates in turn.

2.2 After placing all the plates, press the <Run> icon on the interface to start the program.

2.3 When the program completes, collect the purified bacterial DNA from the elution plate.

2.4 For short term storage, store the samples at 4°C. For long term storage, store the samples at -20°C.