COAST Biostic gDNA extraction using vacuum manifold

Forked from Biostic gDNA extraction using vacuum manifold

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

COAST Extraction Protcol - HMS

MATERIALS

1. Qiagen QIAamp Biostic Bacteremia DNA kit (for bead tubes and reagents only), cat# 12240-50
2. Qiagen DNeasy UltraClean 96 Microbial Kit (for DNA columns only), cat# 10196-4
3. USA Scientific 2.0 ml deep 96-well PP plate, sterile, cat #1896-2110
4. Vacuum manifold for 96 well plate

Sample prep

2h 16m 10s

1 Place tubes on bin of ice for approximately 01:00:00 to thaw sample fully. Record all information present as you move the tubes. 1h

2 Aliquot 100 µL of IRS solution into each well a 2ml deep 96 well plate and seal with foil
Aliquot 1 mL BB solution into each well of a 2ml deep 96 well plate and seal with foil
Aliquot 700 µL EB solution in 8 2mL tubes
Label 96 Powerbead tubes 1-93 (or sample number) and 3 control tubes on lid and side of the tube

DNA isolation

51m 10s

3 Heat MBL solution at 55 °C for 00:05:00 to 00:10:00. Using a multichannel and a reservoir transfer 450 µL MBL solution to 2 mL PowerBead Tube. Add as much sample as possible per tube at a maximum amount of 1.3 mL (650x2). 15m
Create 3 NTCs per extraction batch

4 Vortex for 00:00:10 to mix and place in a 70 °C heat block or water bath for 00:15:00 15m 10s
due to limited heat blocks you have to heat in batches of 48

5 Secure the PowerBead Tube horizontally using the Vortex Adapter tube holder for the vortex (cat. no. 13000-V1-24). Vortex at maximum speed for 00:10:00. 10m
due to limited vortex attachments you have to vortex in batches of 48

6 Centrifuge the PowerBead Tube to pellet debris at 10.000 x g, 00:01:00. 1m

7 Add supernatant to the 2 ml deep well 96 well plate with pre aliquoted 100 µL of Solution IRS, seal, and vortex to mix. Incubate for 00:05:00 at room temperature. Note: Longer incubation in Solution IRS does not affect DNA yield or purity (sample may be incubated up to 10 min in Solution IRS). 5m

8 Centrifuge at 3.750 rpm, 00:03:00. 3m

Note

12/19/22 MB I did this centrifuge step at 3,700 g

9 Transfer the supernatant with a multichannel pipette to 2ml deep 96 well plate containing BB solution. Pipette or pulse vortex to mix. Briefly centrifuge to collect any liquid from the top of the lid.

10 Place 96 well DNA column plate on a vacuum manifold and load 800 µL of lysate to the wells. Turn on vacuum and allow all liquid to pass through. Repeat this step until all the lysate has been loaded onto the DNA column (usually only x2).

11 Wash by adding 500 µL of Solution CB. Turn on vacuum and allow all liquid to pass through.

12 Wash with another 500 µL of Solution CB and Turn on vacuum and allow all liquid to pass through

13 Continue to allow vacuum to dry the DNA Column membrane. Approximately 00:02:00 2m

14 Remove well plate and place over a 96-well semi skirted plate.

15 Elute by adding 50 µL of Solution EB directly in the center of the membrane. Allow sit at room temperature for up to 5 min to maximize the elution.

16 Centrifuge the stacked plates in the centrifuge at 3.750 x g, 00:02:00 to elute DNA. 2m
This step the membrane+ plate looks very suspect I covered the membrane in foil and used foil to secure the membrane to the semi skirt plate.

Note

12/19/22 MB I did this centrifuge step at 3,700 g

17 Discard DNA column plate and aliquot 2 µL of eluted DNA into a second semi skirted 96 well plate.

18 Seal both plates containing 48 µL of eluted DNA and the other containing 2 µL with foil and store at -20 °C

Note: We recommend storing DNA frozen (–20° to –80°C) as Solution EB does not contain EDTA.