BiOstic Bacteremia DNA Isolation Kit Protocol

Purpose: To extract total genomic DNA from environmental swabs

Materials:

MO-BIO BiOstic® Bacteremia DNA Isolation Kit

Vortex Adapter

Microcentrifuge

Copan 552C Flocked Nylon Swabs

Protocol:

1. Placed swab in the MicroBead Tube and added 450 μl of pre-warmed (in 55°C bath) CB1 Solution.
2. Vortexed for 10 seconds and placed in a 70°C heat block for 15 minutes
3. Placed MicroBead Tube into MO BIO Vortex Adapter and vortexed at max speed for 10 minutes
4. Centrifuged MicroBead Tube at 10,000 x g for 1 minute
   * All centrifugation will be the same speed unless otherwise noted
5. Transferred supernatant into new Eppendorf tube
6. Added 100 μl of CB2 Solution and vortex to mix
7. Incubated for 5 minutes at room temperature
8. Centrifuged for 1 minute
9. Transferred supernatant to new Eppendorf tube
10. Added 1000 μl of CB3 Solution
11. Vortexed to mix then briefly centrifuge to collect liquid from top of lid
12. Loaded 600 μl of mix from step 11 into spin filter
13. Centrifuged for 1 minute
14. Repeat steps 12 and 13 until liquid from step 11 has been filtered
15. Transferred the spin filter to a new Eppendorf tube
16. Added 500 μl of CB4 Solution to spin filter
17. Centrifuged for 1 minute
18. Discarded flow through and placed back into the same tube
19. Centrifuged at 13,000 x g for 2 minutes
20. Transferred spin filter to a new Eppendorf tube
21. Added 50 μl of CB5 Solution to center of membrane
22. Let sit at room temperature for 5 minutes
23. Centrifuged at 10,000 x g for 1 minute
24. Discarded the filter and labeled tube as gDNA
25. Stored in -20°C freezer