

User-Developed Protocol

Extraction of DNA from yeast-positive blood culture using the MycXtra[®] Fungal DNA kit

Procedure

The sample should be collected from the blood culture bottle using the appropriate collection device as recommended by the manufacturer.

1. Briefly mix the blood culture by rolling upright between the palms of the hands.
2. Retrieve 1 mL of sample* and transfer to a 1.5 mL microcentrifuge tube(s) and centrifuge for 2 minutes at 10,000 x g.
*Procedure for the bioMerieux BacT ALERT 3D Select system: if not already attached, attach swabable vial adapter(s) (Medimop Medical Projects Ltd. Ref 8072001 are recommended) to the top of the blood culture bottle(s) by pressing down on the device ensuring the rubber seal of the bottle is pierced and the device clicks firmly into place. To retrieve a 1 mL blood sample(s), attach a 1 mL or 3 mL luer lock syringe to the top of the blood culture bottle(s) by gently screwing it into the swabable vial adapter. Invert the bottle and draw out 1 mL of blood culture with the syringe. Note: blood culture bottle is under vacuum pressure, so make sure that the blood culture hasn't been vacuumed back to the bottle when detaching the syringe from the adapter.
3. Avoiding disturbing the red cell sediment, remove and discard approximately 750 µL of the supernatant and resuspend the pellet in the remaining solution.
Note: occasionally, removal of 750 µL of supernatant is not possible due to the large pellet size. In these cases, remove most of the supernatant but leave approximately 100 µL in the tube for resuspending the pellet.
4. Transfer the entire resuspended pellet to 2 mL MycXtra[®] Bead Solution tube.
5. Gently vortex to mix.
6. **Check Solution S1.** If Solution S1 has precipitated, warm the tube in the hand and vortex mix to dissolve.
7. Add 60 µL of Solution S1 to the Bead Solution tube and invert several times or vortex briefly.
8. Add 200 µL of Solution IRS (Inhibitor Removal Solution) to the Bead Solution tube.
9. Secure bead tube(s) horizontally on a vortex adapter plate (available from Myconostica REF: 080-015) connected to a Vortex-Genie 2 (Scientific Industries, Inc.). Vortex at maximum speed for 10 min.
10. Centrifuge the Bead Solution tube(s) at 10,000 x g for 30 s. **CAUTION:** Be sure not to exceed 10,000 x g or tubes may break. Make sure the 2 mL tubes rotate freely in the centrifuge without rubbing.

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11. Transfer 450 μ L of supernatant to a clean micro-centrifuge tube (provided) and discard the Bead tube.
12. Add 250 μ L of Solution S2 to the supernatant and vortex for 5 s. Incubate at 4 – 8°C for 5 min.
13. Centrifuge the tubes for 1 min at 10,000 x g.
14. Avoiding the pellet, transfer entire volume of the supernatant to a clean micro-centrifuge tube (provided).
15. Add 1.1 mL (2 x 550 μ L) of Solution S3 to the supernatant (care is required as the tube is almost full) and mix by inverting.
16. Load approximately 650 μ L onto the spin filter and centrifuge at 10,000 x g for 30 s. Discard the flow through, add the remaining supernatant to the spin filter, and centrifuge at 10,000 x g for 30 s. Repeat until all supernatant has passed through the spin filter. On the final spin, centrifuge at 10,000 x g for 1 min. **Note:** A total of three loadings for each sample processed is required. Discard the flow through.
17. Add 300 μ L of Solution S4 and centrifuge for 30 s at 10,000 x g.
18. Discard the flow through.
19. Centrifuge again for 1 min to remove the last traces of S4 which may inhibit the PCR reaction.
20. Carefully place the spin filter in a new clean tube (provided). Avoid any carry over of Solution S4 onto the spin filter.
21. Add 40 μ L of Solution S5 to the center of the white filter membrane. Leave at room temperature for 2 min.
22. Centrifuge for 30 s at 10,000 x g.
23. Discard the spin filter. DNA in the tube is now ready for use in a PCR application. Store at 2 – 8 °C for up to 5 days, otherwise store DNA frozen at -20°C.
24. Up to 10 blood cultures can be processed with one MycXtra[®] kit; if not all components are used on one occasion then return the kit components to the original box. Do not mix different lots.
25. When processing is complete used kit components should be disposed of according to local health, safety and environmental regulations.

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