

Xploregen Discoveries Pvt. Ltd.

SAMPLE SIZE

- Bacterial culture plateTake a pinch of the culture
 with a loop
- 2. <u>Bacterial broth</u>- Take 2 ml of sample in a 1.5 ml tube and centrifuge at 10,000 rpm for 5 minutes at rt. Discard supernatant

IMPORTANT NOTES

- Wear gloves and lab
 coat when handling the
 buffers
- 2. Add the required volume of ethanol (96-100%) to XBA 4 before use.
- 3. Vortex the beaded vial for 2-3 sec
- 4. Perform all the centrifugation steps at room temperature.
- 5. Gently shake the reagents before using.

KIT CONTENTS (50/100/250)

- 1. XBA1-70ml/140ml/350ml
- 2. XBA2-10ml/20ml/5
- 3. XBA3-30ml/60ml/150ml
- 4. XBA4-30ml/55ml/130ml
- 5. XBA5-3ml/6ml/15ml

BACTERIAL DNA EXTRACTION PROTOCOL

- 1. Add 1400 µl of **XBA 1** to the beaded vial.
- 2. Add the sample in the beaded vial containing XBA 1.
- 3. Horizontal vortex the vial at maximum speed for 15 minutes.
- 4. Centrifuge the tube at 10,000 rpm for 2 minutes at room temperature (RT)
- 5. Transfer 700 µl of supernatant to a sterile 2 ml vial.
- 6. Add 200 µl of **XBA 2** solution and vortex for 5 seconds
- 7. Centrifuge at 10,000 rpm for 2 minutes.
- 8. Transfer 750 µl Supernatant to a clean sterile 2ml vial.
- 9. Add 600 µl of **XBA 3** solution to the supernatant and vortex for 5 seconds.
- 10. Transfer 700 µl of Lysate to the spin column (Do not discard the residual lysate) and Centrifuge at 10,000 rpm for 2 minutes.
- 11. Discard the flow through.
- 12. Repeat the above (10 to 11) steps to collect all the Lysate.(Ensure the entire lysate is processed via the spin
 - column)
- 13. Add 500 μl of **XBA 4** to the spin column and Centrifuge at 10,000 rpm for 2 minutes.
- 14. Discard the flow through.
- 15. Centrifuge the empty spin column for 5 minutes at 10,000 rpm.
- 16. Place the spin column into a sterile 1.5 ml vial.
- 17. Add 30 µl of **XBA 5** (Elution 1) to the center area of spin column and centrifuge for 5 minutes at 10,000 rpm.
- 18. Place the spin column to a new sterile 1.5ml vial.
- 19. Add 25 ul of **XBA 5** (Elution 2) to the center area of spin column and centrifuge for 5 minutes at 10,000 rpm.
- 20. Discard the spin column and store both elution tubes for further processing.

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