Exgene™ Stool SV kit

Cat. No. : 115-150

DNA Isolation from Gram-Positive Bacteria in Stool

Before Starting

- Prepare water bath or heating block to 37 ℃
- Prepare Lysozyme (LYS702, Bioshop, Canada, or equivalent) or Lysostaphin (L7386, SIGMA, USA, or equivalent)
- Prepare absolute ethanol
- All centrifugation should be performed at room temperature
- If precipitation is observed in Buffer FL, heat the bottle in 56 ℃ water bath to dissolve completely.

Ordering Information

106-901	Buffer GP	60 ml

Just Before Use : Preparation of Enzyme Mixture

Resuspend the appropriate amount of enzyme with Buffer GP just before use. Enzyme mixture should be stored at -20 ℃ (or bel) in small aliquots; ideally, single use per aliquot. Once aliquots have been frozen and thawed, do not reuse but discard.

Lisozyme (LYS702, Bioshop, Canada, or equivalent) : 30 mg/mL
or/and
Lysostaphin (L7386, SIGMA, USA, or equivalent) : 300 µg/mL

* Generally, lysozyme is sufficient to lyse the cell wall for most gram-positive bacterial strains. However, for certain species such as *Staphylococcus*, treatment of lysostaphin (final conc. =300 µg/mL) may be required for efficient lysis instead of (or with) lysozyme.

1. **Add up to 200 mg of stool sample to a 2 ml tube (provided).**
2. **Add 1 ml of Buffer PBS to the tube and vortex for 1 minute or longer until the stool sample becomes a thorough mixture without lumps.**
3. **Incubate at room temperature for 1 min.**
4. **Transfer the supernatant to a new 2 ml tube (provided).**
   Take caution not to co-transfer the debris of heavier particles.
5. **Centrifuge at 10,000 xg for 2 minutes and discard the supernatant.**
   Take caution not to lose the pellet.
6. **Resuspend the pellet thoroughly in 180 µl of the prepared enzyme mixture. Incubate at 37 ℃ for 30 min.**

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DNA Isolation from Gram-Positive Bacteria in Stool (continued)

7. Centrifuge at 10,000 xg for 3 minutes and discard the supernatant.
Take caution not to lose the pellet.

8. Add 1.3 ml of Buffer FL and resuspend the pellet by pipetting.
To enhance the resuspension, pipetting followed by vortexing can be helpful.

9. Incubate at room temperature for 5 min. Centrifuge at 10,000 xg for 5 min.
If possible, move the supernatant to a new 1.5 ml micro centrifuge tube before Step 8.

10. Transfer the supernatant to an EzPass™ filter (white column).

11. Centrifuge at 10,000 xg for 1 min and discard the pass-through.
If the volume of supernatant exceeds 700 ul, repeat Steps 10~11.

12. Transfer the EzPass™ filter to a new 1.5 ml micro centrifuge tube (provided).

13. Add 100 µl of Buffer EB to the EzPass™ filter and incubate at room temperature for 1 min.

14. Centrifuge at 10,000 xg for 1 min.

15. Add 500 µl of Buffer PB to the pass-through and mix well by pipetting.
Transfer the mixture to a mini spin column (type G, green column).

16. Centrifuge at 10,000 xg for 1 min. Discard the pass-through and reinsert the mini spin column back into the same 1.5 ml micro centrifuge tube.

17. Add 500 µl of Buffer NW to the mini spin column and centrifuge at 10,000 xg for 1 min.
Discard the pass-through and reinsert the mini spin column back into the same 1.5 ml micro centrifuge tube.

18. Centrifuge at 10,000 xg for 1 minute to remove residual wash buffer.
Transfer the mini spin column into a new 1.5 ml micro centrifuge tube (provided).
Residual ethanol may interfere with downstream reactions. Take caution at this step to eliminate carryover of Buffer NW.

19. Add 50 µl of Buffer EB to the center of the membrane in the mini spin column and incubate at room temperature for 1 min.
Elution volume can be decreased to 30 µl for high concentration of DNA, but this will generate slight decrease in overall DNA yield. If maximum recovery is preferred or if the starting material contains large amounts of DNA, elution can be done using 200 µl of Buffer EB.

20. Centrifuge at 10,000 xg for 1 min.