

BUGS'n BEADS™ – DNA isolation kit, S-version

50 isolations

Cat. No: GK1-50

Contents: 1 tubes with blue lid: 1.0 ml of Bacterial Binding Beads
2 tubes with white lid: 1.85 ml Lysis Buffer
1 bottles of 50 ml Binding Buffer

Not supplied with the kit: magnet, 70% ethanol, and 96% ethanol

Storage: The contents of this kit should be stored at 4°C.

Preparations: Allow the contents of the kit come to room temperature before use.
Resuspend the beads before use.

DNA isolation for PCR amplification:

1. Add 20 µl (7,5 mg/ml) Bacteria Binding Beads and 800 µl B&W Buffer to a 1.5 ml microcentrifuge tube.
2. Add 100 µl overnight culture, mix by pipetting gently and leave the tube at room temperature for 5 min.
3. Place the tube in a magnetic separator. Allow the bacteria/bead complex to move to the side of the tube. Carefully discard the supernatant by pipetting without touching the beads.
4. Separate the magnet from the tube. Add 50 µl Lysis Buffer, mix by shaking the tube briefly (do not invert the tube), and incubate at 80°C for 5 min. Keep lids closed.
5. Add 150 µl refrigerated 96% ethanol and mix by shaking. Continue the incubation for 5 min at room temperature.
6. Recombine the magnet and the tube. Invert magnet with the tube gently 2 times to collect all the beads. Carefully pipette off, also from lid, and discard the supernatant.
7. Again separate the magnet from the tube. Add 1ml of 70% ethanol, close lids and mix to evenly distribute the DNA/bead complex in the solution.
8. Recombine the magnet and the tube. When the supernatant clears, invert the tube again and remove as much as possible of the supernatant.
9. Repeat washing step 7 and 8. Before continuing to step 10 make sure all ethanol is removed, also in lids.
10. Resuspend the bead/DNA complex in 30 µl sdH₂O. Incubate the tube at 80°C for 10 min to remove residues of ethanol. Use a heating block, and keep tube lids open.
11. Use 1– 15 µl of the bead/DNA sample in one 50 µl PCR.

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Notes

- It is important that the DNA/bead sample is washed properly with ethanol so that **crystals** formed by the lysis buffer are **removed** during the washing steps (step 7-9).
- Also the ethanol used in the procedure needs to be completely removed; ethanol present in the PCR will result in blank samples.

Gram positive strains

- Include 15 mM CaCl₂ in enrichment media.
- Increase incubation time with lysis buffer to 10 min.

Gram negative strains

- Include 10 mM MgCl₂ in enrichment media.

Yield

- It is possible to increase the yield by using more Bacteria Binding Beads.
- If the sample is expected to have a high density of bacteria, reduce the volume of your sample and compensate with more B&W Buffer.
- If the sample is expected to have a low density of bacteria, increase the volume of your sample.
- The sample may be rotated during the incubation (step 2) to increase the yield.
- The interaction time between the bacteria and the beads may be increased (step 2).

General conditions

- Several types of lactic acid bacteria (e.g. *Streptococcus*, *Lactococcus*, *Lactobacillus*) require prolonged incubation with beads. A few hours or an overnight incubation at room temperature is recommended.