

Troubleshooting

Lack of PCR product

- Make sure that no crystals are present in the POWERlyse before use.
- Make sure that all the ethanol is evaporated according to the procedure; ethanol present in the PCR will result in blank samples. Check that the heating block actually holds 85°C.
- Make sure the lysis step didn't last for more than 5 min.

Low sensitivity

- Increase the sample volume into the procedure to as much as 700µl, the volume of Binding Buffer must then be reduced accordingly.
- The template volume in the PCR was not optimal. Try to reduce or increase the template volume (step 14).
- Mixing or rotating during the bacterial binding step may improve sensitivity.

General guidelines for PCR

Genpoint strongly recommends running the PCR for 40 cycles. Make sure the primers have been tested beforehand and are of satisfactory quality for amplification of small quantities of DNA. Primer pairs available from Genpoint have been thoroughly tested and are recommended for efficient analysis of their intended species. It is recommended to use a commercially available mastermix for the PCR. Use negative and positive control of species DNA, contact us for further suggestions if needed.

Safety information and Warranty

The POWERlyse solution contains potentially harmful components. Suitable laboratory clothing, gloves and safety goggles should be worn when using the kit. A separate Material Safety Data Sheet is available from Genpoint AS. In case of liquid from the POWERlyse is spilled, clean with copious amounts of water and a laboratory detergent. Genpoint AS guarantees the performance of this kit as described in the product literature. Genpoint AS reserves the right to alter, or modify the product to improve the performance. Genpoint's liability is limited in all events to the price of the products sold by Genpoint AS. In no event shall Genpoint AS be liable for any incidental, consequential or special damages.

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BUGS'n BEADS™ version C

For general purposes for the isolation of bacterial DNA

Cat. No: GK2-100,

100 isolations, store at 4°C

Kit Contents

Bacteria binding beads	2 x 1.0 ml (red lid)
POWERlyse solution	3 x 1.7 ml (white lid)
B&W buffer	2 x 36 ml

Components required but not supplied

Magnetic separator (available from Genpoint AS)
Ethanol, 96% and 70%
1% BSA

Intended use

BUGS'n BEADS™ is used to isolate bacteria and subsequently the bacterial DNA from a variety of matrixes, for the purpose of analyzing the DNA. The end product following the entire procedure is nucleic acids of sufficient quality to enable nucleic acid amplification.

The BUGS'n BEADS™ version C is suitable for isolation of clinically related pathogens. Please see www.genpoint.com for an updated list of bacteria that will bind to version C.

Before starting

- Read the entire protocol before starting.
- Allow the contents of the kit to come to room temperature before use.
- Crystals present in the POWERlyse solution must be dissolved by heating (37°C for a few minutes, can alternatively be stored at RT).
- For PCR, make up dH₂O with required amount of BSA (See table 2).
- It is recommended to work with maximum 10 samples at the time.
- Resuspend the beads by shaking vigorously before use.

Preparation of bacterial DNA from samples

1. Mix 20 µl Bacteria binding beads and 600 µl Binding buffer for each sample to be analyzed. (See table 1, next page).
2. For each analysis, aliquot 620 µl of the bead mixture into 1.5 ml microcentrifuge tubes that fit your magnetic rack.
3. Add 300 µl of your sample and mix gently by pipetting.
4. Incubate at room temperature (RT) for 5-15 min from last sample prepared.
5. Place the tubes in a magnetic separator. Allow the beads to be drawn to the side of each tube (about 2 minutes). Carefully discard the supernatant by pipetting, while keeping the tube in the magnetic separator. Avoid removing beads.
6. Separate the tubes from the magnet. Make sure that there are no crystals in the POWERlyse solution, invert the POWERlyse tube to mix the contents. Add 50 µl POWERlyse to each sample tube, mix well by shaking the tube briefly.
Leave the lysis for approximately one minute from addition to the last tube, before proceeding to step 7.
7. Add 150 µl 96% ethanol. Shake vigorously or vortex to resuspend the beads.
8. Incubate for 5 min at RT.
9. Reassemble the tubes with the magnet. Invert the magnet with tubes gently, twice, to collect all the beads (about 2 minutes). Carefully pipette off from the lid first and then the tube, make sure all liquid is removed and discard the supernatant.
10. Again separate the tubes from the magnet. Add 1 ml of 70% ethanol, close lids and mix vigorously to resuspend the beads in the solution.
11. Reassemble the tubes with the magnet and leave for about 2 minutes. When the supernatant is completely clear, invert the tubes once, leave for another minute and remove all the supernatant by pipetting.
12. Repeat washing step 10 and 11. Before continuing to step 13 make sure all ethanol is removed, also in lids. If needed use a smaller pipette tip to remove the remaining liquid. Any remaining ethanol at this point will potentially inhibit a PCR.
13. Separate the tubes from the magnet and resuspend the beads in 50 µl dH₂O containing BSA for a final concentration of 0,02% in the PCR reaction (see table 2). For amplification methods other than PCR, use the buffer provided/recommended by the manufacturer.
14. Incubate the tubes at 85°C for 10 min. with open lids to remove residual ethanol. Make sure that the heating block actually holds 85°C.
15. Reassemble the tubes with the magnet and let the beads collect on the side of the tubes (at least 2 minutes), transfer the supernatant either into a clean tube or directly into the PCR reaction tube. Use approximately 5-20 µl supernatant in a 50 µl final PCR volume .

Table 1, Mix of Bacteria binding beads and Binding buffer

Samples	1	5	10	20
Beads	20 µl	110 µl	220 µl	440 µl
Buffer	600 µl	3,3 ml	6,6 ml	13,2 ml

The calculation for more than 1 sample includes 10% excess volume.

Table 2, BSA/dH₂O mix for elution

For a final conc. of 0.02% BSA in a PCR reaction, use a 1% solution of BSA, and dilute according to table below.

Template in 50 µl PCR	Template in 25 µl PCR	For 10 samples, 600 µl resuspension buffer
10 µl	5 µl	60 µl 1% BSA + 540 µl dH ₂ O
15 µl	7,5 µl	40 µl 1% BSA + 560 µl dH ₂ O
20 µl	10 µl	30 µl 1% BSA + 570 µl dH ₂ O
30 µl	15 µl	20 µl 1% BSA + 580 µl dH ₂ O