Bacterial/ Fungal Lysis Buffer

Product No: K.BUF-9103-50ml

This buffer allows crude nucleic acid extraction from pure isolate of bacterial/ fungal sample. Pure isolate can be from agar culture or cell pellet from liquid culture. This buffer requires addition of Proteinase K enzyme (not provided). The extracted crude DNA is suitable for PCR amplification of 16S rRNA full length gene or ITS gene for Sanger sequencing. It is a solution-based extraction method that utilizes high salt to extract crude nucleic acid for PCR applications.

Storage and Stability

Store the product at room temperature (21 °C – 25 °C). The product expiration date is printed on the label.

Materials Supplied by Users

- ✓ Thermo block, set at 56 °C
- ✓ Centrifuge at 14,000 – 16,000 × *g*
- ✓ Sterile nuclease-free 1.5 mL micro-centrifuge tubes (2x units per sample)
- ✓ Sterile nuclease-free pipette and pipette tips
- ✓ Isopropanol
- ✓ 70% ethanol
- ✓ Proteinase K solution (20 mg/mL)
- ✓ TE buffer

Protocol

Lysis

1. Sample:
   a) Cut agar: Place 0.5 cm x 0.5 cm of bacterial/ fungal cut agar into a new 1.5 mL micro-centrifuge tube.

   OR

   b) Transfer 1 mL bacterial/fungal culture into a new 1.5 mL micro-centrifuge tube. Centrifuge at 14,000 x *g* for 1 minute to collect cell pellet. Discard the supernatant.

2. Add 500 µL of **Bacterial/ Fungal Lysis Buffer** into 1.5 mL micro-centrifuge tube that contains the recommended sample size of bacteria/fungus.
   Tips:
   - ✓ *1 mL filter pipette tip is recommended to be used to add Lysis Buffer.*
   - ✓ *Ensure the entire sample is 100% submerged into Lysis Buffer.*
   - ✓ *Handle each sample one at a time to avoid cross contamination.*

3. Add 3 µL of **Proteinase K solution**. Vortex to mix and spin down briefly.

Lysis	4. Incubate at 56 °C according to incubation time below: a) Bacterial : 30 minutes or overnight (optional) b) Fungal : Overnight 5. Centrifuge the lysate at 14,000 – 16,000 × *g* for 10 minutes.
Washing	6. Transfer ~ 500 µL of supernatant to a new 1.5 mL micro-centrifuge tube, which contains 500 µL of **isopropanol**. Invert the tube several times to mix gently. 7. Centrifuge at 14,000 – 16,000 × *g* for 10 minutes and discard the supernatant. 8. Add 1 mL of **70% ethanol**. Centrifuge again at 14,000 – 16,000 × *g* for 5 minutes and discard the supernatant. *Tip:* 70% ethanol should be prepared freshly or less than a week. 9. Air dry the pellet for 3 minutes.
Elution	10. Re-suspend the dried DNA pellet with 50 µL **TE Buffer** and incubate at 56 °C for < 1 hour. *Tip: If necessary, increase the elution volume using* not more than 100 µL TE Buffer *to dissolve the DNA pellet completely.* 11. Measure the Optical Density (OD) reading using spectrophotometer. Dilute the nucleic acid to 15 – 25 ng/µL. Use 2 µL of this diluted nucleic acid as DNA Template for PCR.

Please contact us at https://base-asia.com/contact/ for more information.