PURO Bacteria

For isolation of genomic DNA from bacteria

PB-L
Productos
Bio-Lógicos

http://www.pb-l.com.ar

PURO-Bacteria

Cat. N° SA07

Kit Contents

Contents	SA07-01 50 preps	SA07-02 100 preps
Buffer BR	15 ml	30 ml
Buffer BA	15 ml	30 ml
Buffer BB	6 ml (+ 24ml de EtOH 96%)	12 ml (+ 48ml de EtOH 96%)
Buffer BC	20 ml	40 ml
Proteinase K	1 ml	2 × 1 ml
Spin Colum	50	100
Collection Tubes 2 ml	50	100
Handbook	1	1

v.1

Additional equipment and reagents required

Phosphate buffered saline (PBS)
Microcentrifuge
RNAsea A (10 mg/ml)
Ethanol (96 - 100%)
Lysozyme

Storage

PURO-Bacteria can be stored dry at room temperature (15 - 25 °C) for up to 12 months without showing any reduction in performance and quality. For longer storage, the kit can be stored at 2 - 8 °C.

Introduction

PURO Bacteria is based on silica membrane technology and special buffer system for many kinds of sample's gDNA extraction. The spin column made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation processing completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Purified DNA is eluted in low-salt buffer or water, ready for use in downstream applications.

DNA purified by PURO Bacteria is highly suitable for restriction analysis, PCR analysis, Southern blotting and cDNA library.

Yield of Genomic DNA with PURO Bacteria Kit

Source	DNA Yield
Bacterial culture (10^6 - 10^8 cells)	5 - 20 µg

Important Notes

1. Repeated freezing and thawing of stored samples should be avoided, since this leads to reduced DNA size.
2. If a precipitate has formed in Buffer BA, warm buffer to 56 °C until the precipitate has fully dissolved.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15 - 25 °C).

Protocol

Ensure that Buffer BB has been prepared with appropriate volume of ethanol (96 - 100%) as indicated on the bottle and shake thoroughly.

1. Pipet 1 - 5 ml bacterial culture suspension in a centrifuge tube by centrifuging for 1 min at 10,000 rpm (~11,500 ×g). Discard supernatant.
2. Add 200 µl **Buffer BR**. Mix thoroughly by vortex.
   Note: For difficult-broken Gram-positive bacteria, you can skip Step 2 and add lysozyme. The specific method is: add 180 µl enzymatic lysis buffer (20 mM Tris-Cl, pH 8.0; 2 mM sodium EDTA; 1.2% Triton® X-100; add lysozyme to 20 mg/ml immediately before use) and incubate for at least 30 min at 37 °C. *Optional: RNAse treatment of the sample. Add 4 µl of RNAse A (10 mg/ml), mix by vortex and incubate for 5 min at room temperature (15 - 25 °C).*
3. Add 20 µl **Proteinase K**. Mix thoroughly by vortex.
4. Add 220 µl Buffer **BA** to the sample, mix thoroughly by vortex and incubate at 70 °C for 10 min to yield a homogeneous solution. Briefly centrifuge the 1.5 ml centrifuge tube to remove drops from the inside of the lid.
   Note: Do not add Protease K directly to Buffer BA.
5. Add 220 µl ethanol (96 - 100%) to the sample and mix thoroughly by vortex for 15 s. A white precipitate may form on addition of ethanol. Centrifuge the 1.5 ml centrifuge tube at 10,000 ×g for 2 min to remove drops from the inside of the lid.

6. Pipet the mixture from step 5 into the Spin Column (in a 2 ml collection tube) and centrifuge at 12,000 rpm (~13,400 ×g) for 30 s. Discard flow-through and place the spin column into the collection tube.
7. Add 500 µl **Buffer BB (prepared with appropriate volume of ethanol 96 - 100%)** to the Spin Column and centrifuge at 12,000 rpm (~13,400 ×g) for 30 s, then discard the flow-through and place the spin column into the collection tube.
8. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 min to dry the membrane completely.

   Note: The residual ethanol of Buffer BB may affect downstream application.
9. Place the Spin Column in a new clean 1.5 ml centrifuge tube and pipet 50 - 200 µl **Buffer BC** or distilled water directly to the center of the membrane. Incubate at room temperature (15 - 25 °C) for 2 - 5 min and then centrifuge for 2 min at 12,000 rpm (~13,400 ×g).

   Note: If the volume of eluted buffer is less than 50 µl, it may affect recovery efficiency. What's more, the pH value of eluted buffer will have some influence in eluting, we suggest choose buffer TE or distilled water (pH 7.0 - 8.5) to elute gDNA. For long-term storage of DNA, eluting in Buffer TE and storing at -20 °C is recommended, since DNA stored in water is subject to acid hydrolysis.
10. A second elution step with a further 50 - 200 µl Buffer BC will increase DNA yield.
11. Volumes of more than 200 µl should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.