Promega

Product Application

GRAM+ bacterial RNA Purification

Isolate high quality, amplifiable RNA from GRAM+ bacteria using the Reliaprep™ RNA Cell Miniprep System

Kit: ReliaPrep™ RNA Cell Miniprep System (Z6012)

Analyses: Quantifluor® quantitation, RT-PCR amplification

Sample Type(s): *Bacillus.Subtilis*

Input: up to $1*10^9$ cells

Materials Required:

- Reliaprep™ RNA Cell Miniprep System (Z6012)
- Lysozyme at 10mg/ml
- Thermomixer

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TM370, available at: **www.promega.com/protocols**

Protocol:

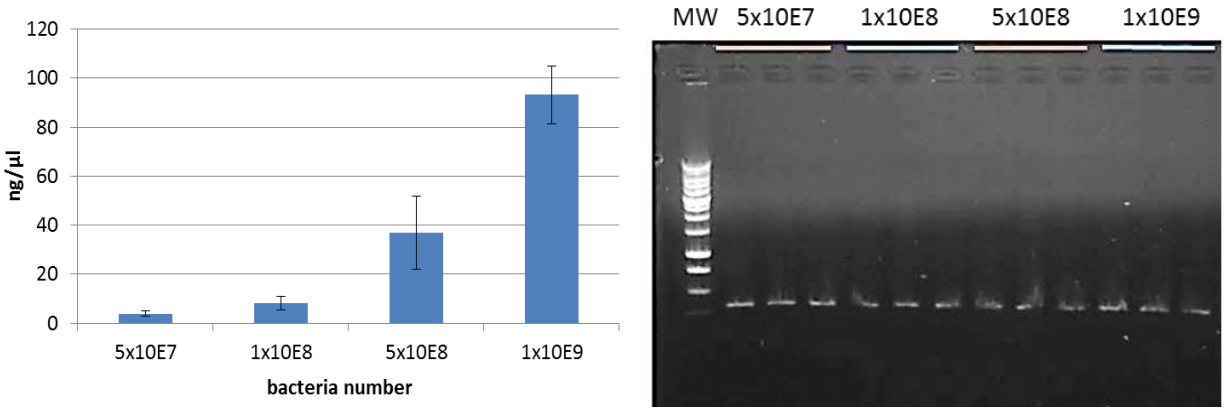
1. Grow a culture of *B Subtilis* overnight at 30°C in a LB medium.
2. Make a 1:10 dilution in LB medium and grow during approx. 2 hours at 30°C until to obtain an O.D. =0.8-1.
3. Put up to $1*10^9$ bacteria in a 1.5ml tube
4. Make the pellet spinning during 2 min at max. speed
5. Add 100 µl of lysozyme and incubate 10 min at 30°C and at 1100 rpm in a thermomixer
6. Add 250 µl BL+TG buffer and vortex 5 sec
7. Disperse the cell pellet and mix well by vortexing
8. Add 85 µl of isopropanol
9. Mix by vortexing 5 seconds
10. Wearing gloves, unpack one Minicolumn, two Collection Tubes and one Elution Tube for each sample. Label each tube and Minicolumn. Place one Minicolumn into a Collection Tube for each sample.
11. Transfer lysate to a Minicolumn in a Collection Tube.Centrifuge at 12,000–14,000 × g for 30 seconds at 20°–25°C
12. Remove the ReliaPrep™ Minicolumn and discard liquid in the Collection Tube
13. Replace the Minicolumn in the Collection Tube. Add 500µl of RNA Wash Solution to the Minicolumn. Centrifuge at 12,000–14,000 × g for 30 seconds. Empty the Collection Tube.
14. Prepare DNase I incubation mix by combining the following amounts of reagent, per sample, in the order listed:
    - 24 µl Yellow Core Buffer
    - 3µl MnCl2, 0.09M

- 3µl DNase I

Mix by gently pipetting; do not vortex. The volumes listed above make enough DNase I mix for a single sample. Multiply this amount by the number of samples to calculate the amount of DNase I mix to prepare.

15. Apply 30µl of DNase I incubation mix to the minicolumn membrane. Incubate for 15min at 20-25°C
16. Add 200µl of Column Wash Solution (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds
17. Add 500µl of RNA Wash Solution (with ethanol added). Centrifuge at 12,000–14,000 × g for 30 seconds. Discard the wash solutions and the Collection Tube.
18. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add 300µl of RNA Wash solution and centrifuge at high speed for 2 minutes.
19. Transfer the ReliaPrep™ Minicolumn from the Collection Tube to an Elution Tube. Add 50µl of Nuclease-Free Water to the Minicolumn membrane. Place the Minicolumn and Elution Tube into a centrifuge with the Elution Tube lid facing to the outside. Centrifuge at 12,000–14,000 × g for 1 minute.

Results:



Left: DNA quantitation using Quantifluor® RNA system (n=3). **Right**: amplification of RNA extracted from B.Subtilis by RT-PCR. MW: molecular weight.