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): *E.Coli*

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:

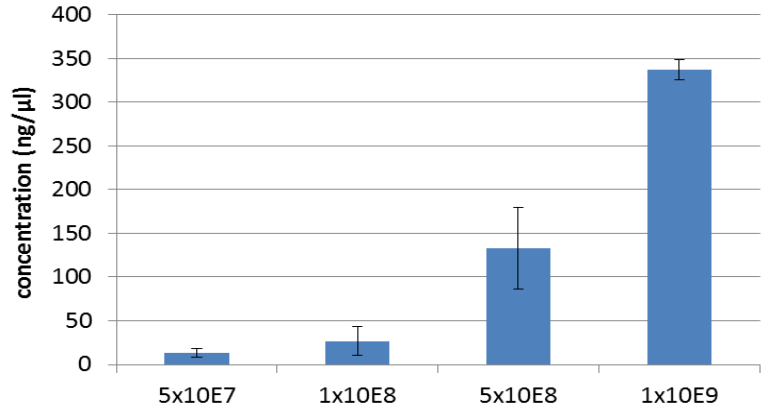
1. Grow an overnight culture of *E.coli* in LB medium at 37°C.
2. Make a 1:10 dilution of the overnight culture. Grow the culture to an OD_{600} approx. 0.8-1 (around 2h) at 37°C and 800 rpm
3. Put up to $1*10^9$ bacteria in 1.5 ml tube
4. Centrifuge at 13000 rpm 2 min to get the pellet
5. Remove the supernatant
6. Add 100 µl of lysozyme at 10mg/ml (preparation of 20ml at 10mg/ml for 20 aliquot of 1 ml : Weight 200 mg of lysozyme and add 20 ml of TE; store at -20°C)
7. Incubate for 5 minutes at room temperature
8. Add 250 µl BL+TG buffer buffer
9. Disperse the cell pellet and mix well by vortexing
10. Add 85 µl isopropanol
11. Mix by vortexing 5 seconds
12. 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.
13. Transfer lysate to a Minicolumn in a Collection Tube.Centrifuge at 12,000–14,000 × g for 30 seconds at 20°–25°C
14. Remove the ReliaPrep™ Minicolumn and discard liquid in the Collection Tube
15. 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.
16. Prepare DNase I incubation mix by combining the following amounts of reagent, per sample, in the order listed:
    - 24µl Yellow Core Buffer

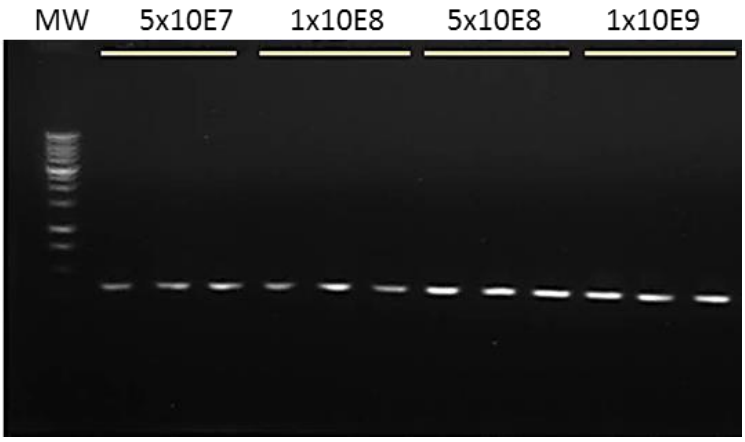
- 3µl MnCl2, 0.09M
- 3 µl DNase I

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.

17. Apply 30µl of DNase I incubation mix to the minicolumn membrane. Incubate for 15min at 20-25°C
18. Add 200µl of Column Wash Solution (with ethanol added) to the Minicolumn. Centrifuge at 12,000–14,000 × g for 15 seconds
19. 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.
20. Place the ReliaPrep™ Minicolumn into a new Collection Tube. Add 300µl of RNA Wash solution and centrifuge at high speed for 2 minutes.
21. 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:





Top: DNA quantitation using Quantifluor® RNA system (n=3). **Bottom**: amplification of RNA extracted from *E.coli* by RT-PCR. MW: molecular weight.