BECKMAN COULTER
Life Sciences

Isolation of RNA from Bacteria using RNAdvance Tissue Kit

Please reference current RNAdvance Tissue protocol for product information (A32646, A32649, A32645)

RNA isolation from bacterial cultures is a standard extraction method for determining genes expressed in the culture. Downstream assays usually require high-quality RNA, and the RNA extraction described below results in good yields of high-quality RNA.

Purpose

High-quality RNA from bacterial cultures is critical for obtaining meaningful gene expression data. RNA preparations with lower quality can yield differences in relative gene expression ratios leading to errors in the quantification of transcript levels. This protocol optimizes RNA isolation from a wide range of bacteria using RNAdvance Tissues with lysozyme. Lysozyme is not needed when extracting from gram-negative bacteria.

Material Used

Material	Part Number	Supplier
1.2 mL 96-well plate	AB1127	ThermoFisher Scientific
Lysozyme from chicken egg white	L6876	Sigma-Aldrich
100% Ethanol (Molecular Grade)	AB00138	AmericanBio
Nuclease-free water (Molecular Grade)	AM9932	ThermoFisher Scientific
100 % Isopropanol (Molecular Grade)	AB07015-01000	AmericanBio
7 Bar Magnet for 96-Well Plate	771MWZM-1ALT	V&P Scientific
RNAdvance Tissue Kit	A32646, A32649, A32645	Beckman Coulter

Protocol

1. **Sample Preparation**
    a. Transfer **150 µL** of bacterial overnight culture with an OD < 6.5 (or equivalent cell amount) to a well in a 1.2 mL 96-well plate
    b. Pellet by spinning for **5 minutes** at **3000 x g**
    c. **Remove and discard the supernatant without disrupting the pellet**
2. **Lysis**
    a. Add **400 µL** of **Lysis Buffer LBE** to the plate
    b. Add **20 µL** of **Proteinase K** to the plate
    c. Add **4 µL** of **Lysozyme** (100 mg/mL) to the plate
    d. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    e. **Incubate** the plate for **30 minutes** at **37°C**

3. **Bind**
    a. Vortex the bottle of **Bind Buffer BBC** to fully resuspend the beads
    b. Combine **80 µL Bind Buffer BBC** and **320 µL Isopropanol** for Bind Solution
    c. Add **400 µL** of **Bind Solution** to the plate
    d. **Incubate** the plate for **5 minutes** at **room temperature**
    e. Place the plate on a **magnet** for **15 minutes** (or until the supernatant is clear)
    f. Remove and discard the supernatant without disrupting the beads
    g. Remove the plate from the magnet

4. **Wash**
    a. Add **800 µL** of **Wash Buffer WBD** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **7 minutes** (or until the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet

5. **Ethanol Wash**
    a. Add **800 µL** of **70% Ethanol** to the plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. Place the plate on a **magnet** for **4 minutes** (or until the supernatant is clear)
    d. Remove and discard the supernatant without disrupting the beads
    e. Remove the plate from the magnet

6. **DNase Treat**
    a. Add **100 µL** of **DNase solution** to the plate
        i. DNase solution is prepared as follows
            a. **10 µL** of **10x DNase Buffer**
            b. **10 µL** of **DNase I**
            c. **80 µL** of **nuclease-free water**
    b. **Incubate** the plate for **1 min** at **room temperature**
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    d. **Incubate** the plate for **15 minutes** at **37°C**

7. **Rebind**
    a. Add **550 µL** of **Wash Buffer WBD** to plate
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed
    c. **Incubate** the plate for **5 minutes** at **room temperature**
    d. Place the plate on a **magnet** for **6 minutes** (or until the supernatant is clear)
    e. Remove and discard the supernatant without disrupting the beads

8. **Ethanol Washes (Leave samples on the magnet for the following steps)**
    a. Add **600 µL** of **70% ethanol** to plate
    b. Place the plate on the magnet for **1 minute**
    c. Remove and discard the supernatant without disrupting the beads
    d. Repeat steps 8.a-8.c for a total of **3 washes**
    e. Remove the plate from the magnet

9. **Elute**

   a. Add **40 µL** of **nuclease-free water** to plate

   b. **Incubate** the plate for **2 minutes** at **room temperature**

   c. Place the plate on a **magnet** for **2 minutes** (or until the supernatant is clear)

   d. Remove and **Save** the supernatant without disrupting the beads

Example Data

Escherichia coli, Bacillus subtilis, and *Streptococcus saliverius* were grown overnight in LB media at 37°C. The *E. coli* grew to an OD of 6.59, the *B. subtilis* to an OD of 4.15, and the *S. saliverius* to an OD of 0.94. RNA was extracted from 150 µL of those overnight cultures as described above. The yield was measured by absorbance at 260 on a NanoDrop (Thermo Fisher Scientific), and integrity was measured using an Agilent Tapestation.

The amount of RNA corresponds to the amount of bacterial input, with larger numbers of bacterial cells resulting in higher yields (Figure 1). The RNA was high quality, with a RIN value of 8.4 for *E. coli*, 9.4 for *B. subtilis,* and 7.4 for *S. saliverius* (Figure 2). This protocol worked well for a wide range of bacterial species and provided high-quality RNA.

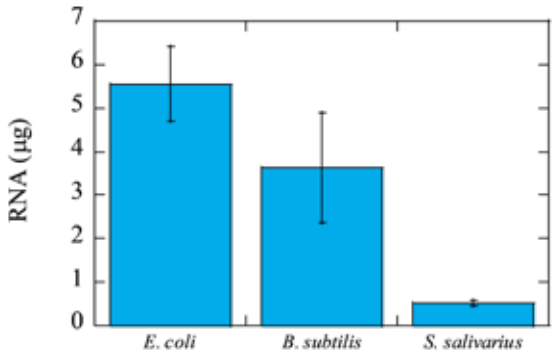

Figure 1. RNA Yield. The yield was measured by absorbance at 260. Error bars represent the standard deviation of three replicates.

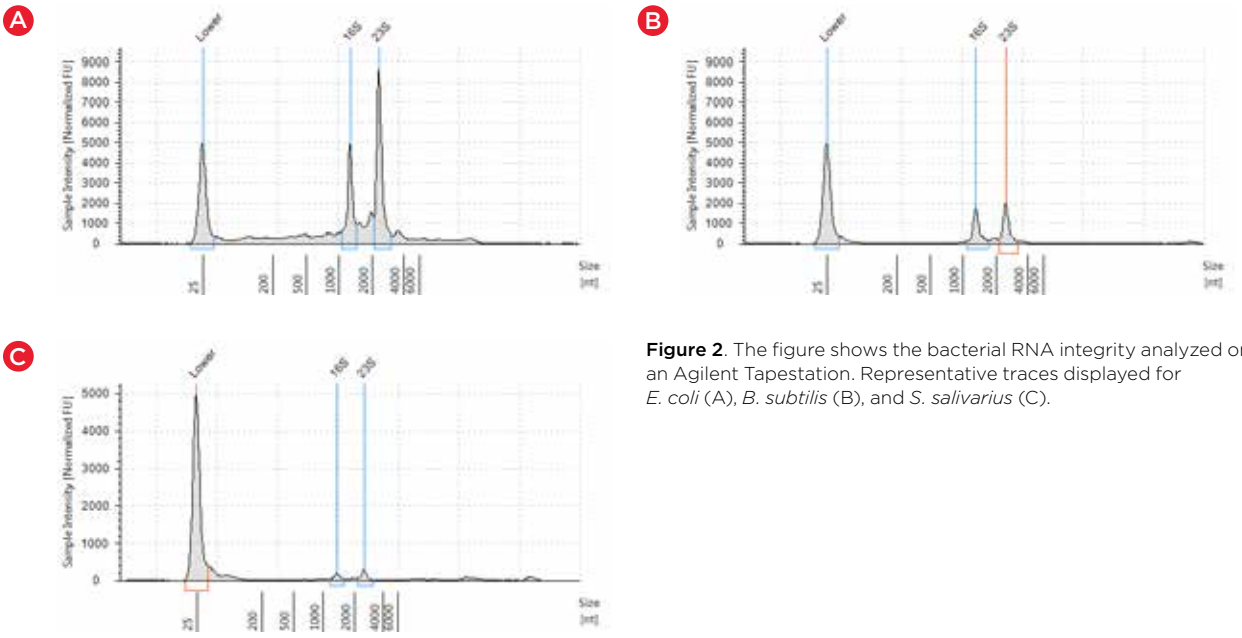

Figure 2. The figure shows the bacterial RNA integrity analyzed on an Agilent Tapestation. Representative traces displayed for *E. coli* (A), *B. subtilis* (B), and *S. salivarius* (C).

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