RNA Isolation from Mucoid Bacteria with RNAdvance Tissue Kit

Please reference the current RNAdvance Tissue protocol for product information (Part Number A32646, A32649, A32645).

Researchers working on mucoid bacteria (i.e. Pseudomonas aeruginosa), bacteria producing excessive exopolysaccharides, or cystic fibrosis infection models may use this protocol.

Purpose

Several bacterial species make extensive amounts of exopolysaccharide (EPS), which can significantly increase the viscosity of samples and also lead to co-elution of polysaccharides with DNA. This increased viscosity can clog columns and prevent bead settling if not considered in the RNA purification process. This protocol significantly reduces viscosity due to the presence of exopolysaccharides and decreases the amount of polysaccharide in the final eluate.

Materials Used

Material	Part Number	Supplier
1.2mL 96-well plate	AB1127	ThermoFisher Scientific
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
PEG EDTA (5% PEG, 0.25 M EDTA)	AB01500, AB00502	AmericanBio
EDTA (0.5 M)	AB00502	AmericanBio
DNase I	AM2222	ThermoFisher Scientific

Protocol

1. **Prepare Sample**
   a. Grow cells up overnight in LB media.
   b. Spin down 200 – 500 µL cell culture at 3000 x g for 5 min.
   c. Remove and discard the supernatant.
2. **Rinse cells**
   a. **Resuspend** cells in **800 µL PEG EDTA** (5% PEG, 0.25 M EDTA).
   b. Transfer samples to a 96 well plate.
   c. **Spin** cells down **3000 x g** for **10 min**.
   d. Remove and discard the supernatant.

3. **Lysis**
    a. **Resuspend** cell pellet in **380 µL RNAdvance Tissue Lysis buffer, 20 µL Proteinase K**, and **100 µL EDTA** (0.5 M).
    b. **Incubate** for **30 min** at **37˚C** with 400 rpm shaking.
4. **Bind**
    a. Add **50 µL 100% ethanol** to the plate.
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    c. Add **450 µL bind/isopropanol** (90 µL bind buffer and 360 µL isopropanol per sample) to the plate.
    d. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    Note: Bead clumps will form as solution is mixed.
    e. **Incubate** for **5 min** at **room temperature**.
    f. Place on **magnet** for **10 min** (or until supernatant is clear).
    Note: Slightly longer incubation time to ensure that beads settle; lysate will still have slight color.
    g. Remove and discard the supernatant.
5. **Wash 1**
    a. Add **800 µL Wash buffer** to the plate.
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    Solution should turn brown, but clumps will still be present.
    c. Place on **magnet** for **5 min** (or until supernatant is clear).
    d. Remove and discard the supernatant.
6. **Wash 2**
    a. Add **800 µL 70% ethanol** to the plate.
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    c. Place on **magnet** for **2 min** (or until supernatant is clear).
    d. Remove and discard the supernatant.
7. **DNase Treatment**
    a. Add **100 µL DNase mix** (10 µL DNase, 10 µL DNase buffer, and 80 µL NFH_2O) to the plate.
    b. **Incubate** for **1 min** at **room temperature**.
    c. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    d. **Incubate** for **15 min** at **37˚C**.
8. **Rebind RNA**
    a. Add **550 µL Wash buffer** to the plate.
    b. **Mix** by pipetting up and down 10 times, or until thoroughly mixed.
    No clumps should be present after this mix step.
    c. **Incubate** for **4 min** at **room temperature**.
    d. Place on **magnet** for **2 min**.
    e. Remove and discard the supernatant.
9. **Washes 3 – 5**
    a. Add **600 µL 70% ethanol** while on magnet; **do not mix**.
    b. Leave on **magnet** for **2 min**.
    c. Remove and discard the supernatant.
    d. **Repeat** steps a through c for a total of **3 ethanol washes**.
10. **Elution**
    a. Add **40 µL nuclease free water** to the plate.
    b. **Incubate** for **2 min** at **room temperature**.
    c. Place on **magnet** for **2 min** (or until supernatant is clear).
    d. Remove and **Save** the supernatant without disrupting the beads.

Sample	Conc. (ng/µL)	A260/A280	A260/A230	total µg
OD 2	596.1	2.01	2.10	23.8
OD 2	448.2	1.98	1.80	17.9
OD 2	591.3	1.98	1.75	23.7
OD 3	410.5	2.00	1.90	16.4
OD 3	366.1	2.06	2.16	14.6
OD 3	537	2.01	2.00	21.5
OD 4	479.6	1.94	1.84	19.2
OD 4	348.7	2.04	1.75	13.9
OD 4	429.3	1.99	1.72	17.2

Table 1. RNA Yield and Purity from Individual Replicates.

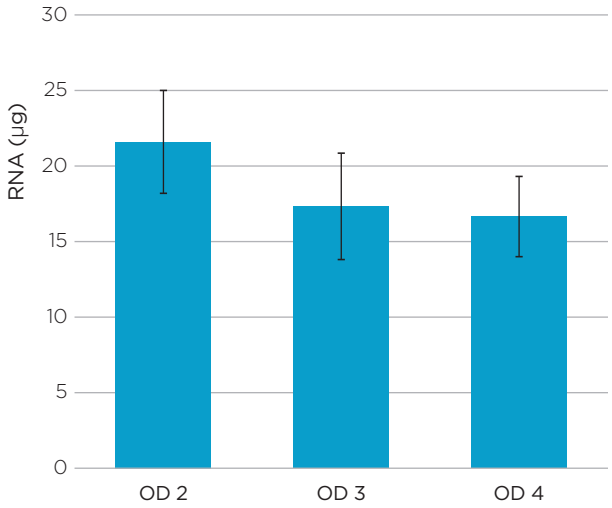

Figure 1. Yield of RNA from varying concentrations of *P. aeruginosa* as measured by absorbance at λ_{260}. Error bars represent the standard deviation of three technical replicates.

Example Data

Pseudomonas aeruginosa strain PA14 was grown overnight in LB media (1 mL) at 37°C with 250 rpm shaking. Cultures had reached approximately OD 8 at the time of extraction. The protocol was followed as written above with 245, 368, and 490 µL of cell culture which correspond to 1 mL of cell culture at ODs of 2, 3, and 4.

The protocol above significantly reduced viscosity in the sample after the lysis step, reducing the bead settling time during the bind step and leading to a cleaner looking pellet without beads binding to strings of exopolysaccharide. RNA was eluted in approx. 350 - 600 ng/µL concentrations, with total yields of greater than 15 µg (Figure 1). Yield was measured by absorbance at λ_{260}. This protocol removed protein contamination, as seen by A260/A280 values above 1.95, and only contained low levels of polysaccharides as shown by A260/A230 values greater than 1.70 (Table 1).

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