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**

<table>
<thead>
<tr>
<th>Material</th>
<th>Part Number</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2mL 96-well plate</td>
<td>ABI127</td>
<td>ThermoFisher Scientific</td>
</tr>
<tr>
<td>100% Ethanol (Molecular Grade)</td>
<td>AB00138</td>
<td>AmericanBio</td>
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<td>Nuclease-free water (Molecular Grade)</td>
<td>AM9932</td>
<td>ThermoFisher Scientific</td>
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<tr>
<td>100% Isopropanol (Molecular Grade)</td>
<td>AB07015-01000</td>
<td>AmericanBio</td>
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<tr>
<td>7 Bar Magnet for 96-Well Plate</td>
<td>771MWZM-1ALT</td>
<td>V&amp;P Scientific</td>
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<td>RNAdvance Tissue Kit</td>
<td>A32646, A32649, A32645</td>
<td>Beckman Coulter</td>
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<td>PEG EDTA (5% PEG, 0.25 M EDTA)</td>
<td>AB01500, AB00502</td>
<td>AmericanBio</td>
</tr>
<tr>
<td>EDTA (0.5 M)</td>
<td>AB00502</td>
<td>AmericanBio</td>
</tr>
<tr>
<td>DNase I</td>
<td>AM2222</td>
<td>ThermoFisher Scientific</td>
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</table>

**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 RNAAdvance 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 H2O) 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.
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).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. (ng/µL)</th>
<th>A260/A280</th>
<th>A260/A230</th>
<th>total µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>OD 2</td>
<td>596.1</td>
<td>2.01</td>
<td>2.10</td>
<td>23.8</td>
</tr>
<tr>
<td>OD 2</td>
<td>448.2</td>
<td>1.98</td>
<td>1.80</td>
<td>17.9</td>
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<tr>
<td>OD 2</td>
<td>591.3</td>
<td>1.98</td>
<td>1.75</td>
<td>23.7</td>
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<tr>
<td>OD 3</td>
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<td>2.00</td>
<td>1.90</td>
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<tr>
<td>OD 3</td>
<td>366.1</td>
<td>2.06</td>
<td>2.16</td>
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<td>OD 3</td>
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<td>2.01</td>
<td>2.00</td>
<td>21.5</td>
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<tr>
<td>OD 4</td>
<td>479.6</td>
<td>1.94</td>
<td>1.84</td>
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<tr>
<td>OD 4</td>
<td>348.7</td>
<td>2.04</td>
<td>1.75</td>
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<tr>
<td>OD 4</td>
<td>429.3</td>
<td>1.99</td>
<td>1.72</td>
<td>17.2</td>
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</tbody>
</table>

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.