### Aurum™ Total RNA Mini Kit Vacuum Protocol Overview

<table>
<thead>
<tr>
<th>Cultured cells</th>
<th>Bacterial cells</th>
<th>Yeast cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adherent</strong></td>
<td>Rinse vessel with PBS, aspirate.</td>
<td>Transfer up to $2 \times 10^6$ cells into a capped 2 ml tube. Centrifuge at maximum speed 1 min. Decant supernatant.</td>
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<tr>
<td><strong>Nonadherent</strong></td>
<td>Rinse with PBS. Transfer up to $2 \times 10^6$ cells, centrifuge 2 min. Decant supernatant.</td>
<td>Add 100 µl of 500 µg/ml lysozyme. Pipet up and down. Incubate at room temp. for 5 min.</td>
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</table>

Add 350 µl lysis solution. Pipet up and down 12x.

Add 350 µl 70% EtOH. Pipet up and down.

Add 350 µl 70% isopropyl alcohol. Pipet up and down.

**Homogenized lysate**

**700 µl low stringency wash**

**80 µl DNase I in dilution solution**

**700 µl high stringency wash**

**700 µl low stringency wash**

**80 µl elution solution**

*Refer to manual for detailed protocol.*

### Assemble manifold properly for isolation.

**Transfer lysate to RNA binding column.** Apply vacuum.

**Add 700 µl low stringency wash.** Apply vacuum.

**Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.**

**Add 80 µl diluted DNase I.** Incubate 15 min at room temp. Apply vacuum.

**Add 700 µl high stringency wash.** Apply vacuum.

**Add 700 µl low stringency wash.** Apply vacuum. Spin-purge 2 min into a capless 2 ml tube.

**Place RNA binding column into a 1.5 ml capped tube.**

**Add 80 µl 70°C elution solution onto membrane stack.** Incubate 1 min. Centrifuge 2 min to elute.

**Aurum Total RNA Mini Kit: Cat. #732-6820**

For more information, call Technical Service at 1-800-4BIORAD (1-800-424-6723). Visit us on the Web at www.bio-rad.com
**Animal tissue**

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 20 mg (hard tissue) or 40 mg (soft tissue) to a capped 2 ml tube.

**Plant tissue**

Cut tissue into small pieces (<5 mm).
Grind into fine powder under liquid nitrogen.
Do not let tissue thaw.

Transfer up to 60 mg to a capped 2 ml tube.

Continue with the following steps for all sample types:

1. **Add 700 µl lysis solution.**
   Disrupt vigorously with rotor-stator for 30–60 sec.

2. **Centrifuge lysate at maximum speed for 3 min.**
   Transfer supernatant to a new 2 ml capped tube.

3. **Add 700 µl EtOH (use 60% for animal tissue, 70% for plant tissue) to supernatant.**
   Homogenize with rotor-stator 30 sec.

4. **Assemble manifold properly for isolation.**

5. **Transfer lysate.**
   Apply vacuum.

6. **Add 700 µl low stringency wash.**
   Apply vacuum.

7. **Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.**

8. **Add 80 µl diluted DNase I.**
   Incubate at room temp. 25 min for animal tissue, 15 min for plant tissue. Apply vacuum.

9. **Add 700 µl high stringency wash.**
   Apply vacuum.

10. **Add 700 µl low stringency wash.**
    Apply vacuum. Spin-purge 2 min into 2 ml capless tube.

11. **Place RNA binding column into a 1.5 ml capped tube.**

12. **Add 80 µl 70°C elution solution onto membrane stack.**
    Incubate 1 min. Centrifuge 2 min to elute.

* Refer to manual for detailed protocol.