## Aurum™ Total RNA Mini Kit Spin Format Protocol Overview

### Cultured cells

<table>
<thead>
<tr>
<th><strong>Adherent</strong></th>
<th><strong>Nonadherent</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Rinse vessel with PBS, aspirate.</td>
<td>Rinse with PBS.</td>
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<tr>
<td>Lyse in vessel if # of cells &lt;2 x 10⁶.</td>
<td>Transfer up to 2 x 10⁶ cells, centrifuge 2 min.</td>
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<tr>
<td>Decant supernatant.</td>
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</table>

- **Transfer lysate.**  
  Centrifuge 30 sec. Discard filtrate.

- **Add 700 µl low stringency wash.**  
  Centrifuge 30 sec. Discard filtrate.

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

  - **Add 80 µl diluted DNase I.**  
    Incubate 15 min at room temp. Centrifuge 30 sec. Discard filtrate.

- **Add 700 µl high stringency wash.**  
  Centrifuge 30 sec. Discard filtrate.

- **Add 700 µl low stringency wash.**  
  Centrifuge 1 min. Discard filtrate.  
  Centrifuge additional 2 min.

- **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.

* Refer to manual for detailed protocol.

### Bacterial cells

- **Transfer up to 2.4 x 10⁹ cells into a capped 2 ml tube.**  
  Centrifuge at maximum speed 1 min.  
  Decant supernatant.

- **Add 100 µl of 500 µg/ml lysozyme.**  
  Pipet up and down.

- **Incubate at room temp. for 5 min.**

- **Add 700 µl high stringency wash.**  
  Centrifuge 1 min. Discard filtrate.

- **Add 700 µl low stringency wash.**  
  Centrifuge additional 2 min.

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

- **Add 80 µl lysis solution.**  
  Pipet up and down.

- **Add 250 µl 70% isopropyl alcohol.**  
  Pipet up and down.

### Yeast cells

- **Transfer up to 3 x 10⁷ cells into a capped 2 ml tube.**  
  Centrifuge at maximum speed 1 min.  
  Decant supernatant.

- **Add 1 ml of 50 U/ml lyticase in lyticase dilution buffer.**  
  Pipet up and down.

- **Incubate at room temp. for 10 min.**  
  Centrifuge at 5,000 rpm for 5 min. Discard supernatant.

- **Transfer up to 2.4 x 10⁹ cells into a capped 2 ml tube.**

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

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

- **Add 350 µl 70°C elution solution.**  
  Pipet up and down.

### For more information

### 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.

- Add 700 µl lysis solution.
  - Disrupt vigorously with rotor-stator for 30–60 sec.
- Centrifuge lysate at maximum speed 3 min.
  - Transfer supernatant to a new 2 ml capped tube.
- Add 700 µl EtOH (60% EtOH for animal tissue, 70% EtOH for plant tissue) to supernatant.
  - Homogenize with rotor-stator 30 sec.
- Insert RNA binding column into a 2 ml capless tube.
- Transfer lysate, centrifuge 60 sec.
  - Discard filtrate. Repeat if necessary.
- Add 700 µl low stringency wash.
  - Centrifuge 30 sec. Discard filtrate.
- Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.
- Add 80 µl diluted DNase I.
  - Incubate at room temp. 25 min for animal tissue, 15 min for plant tissue. Centrifuge column 30 sec. Discard filtrate.
- Add 700 µl high stringency wash.
  - Centrifuge 30 sec. Discard filtrate.
- Add 700 µl low stringency wash.
  - Centrifuge additional 1 min.
- 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.

### 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.

- Add 700 µl lysis solution.
  - Disrupt vigorously with rotor-stator for 30–60 sec.
- Centrifuge lysate at maximum speed 3 min.
  - Transfer supernatant to a new 2 ml capped tube.
- Add 700 µl EtOH (60% EtOH for animal tissue, 70% EtOH for plant tissue) to supernatant.
  - Homogenize with rotor-stator 30 sec.
- Insert RNA binding column into a 2 ml capless tube.
- Transfer lysate, centrifuge 60 sec.
  - Discard filtrate. Repeat if necessary.
- Add 700 µl low stringency wash.
  - Centrifuge 30 sec. Discard filtrate.
- Dilute 5 µl reconstituted* DNase I with 75 µl DNase dilution solution.
- Add 80 µl diluted DNase I.
  - Incubate at room temp. 25 min for animal tissue, 15 min for plant tissue. Centrifuge column 30 sec. Discard filtrate.
- Add 700 µl high stringency wash.
  - Centrifuge 30 sec. Discard filtrate.
- Add 700 µl low stringency wash.
  - Centrifuge additional 1 min.
- 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.

* Refer to manual for detailed protocol.