G 50
Motor-driven
Tissue grinder
User Guide
Content

3 Preface

4 Chapter 1 Plant tissue

4 Efficiency of G50 for different plant tissues’ grinding
6 Efficiency comparison of different methods for grinding the rice leaves
7 Quality comparison of the RNA extracted from the rice leaves grinded through different methods

8 Chapter 2 Animal tissue

8 Efficiency of G50 for different animal tissues’ grinding
10 Efficiency comparison of different grinding methods for the longissimus muscle

11 Chapter 3 Microbes

11 Efficiency comparison of different methods for grinding the fungus hypha
12 Quality comparison of the RNA extracted from the fungus hypha grinded through different methods

13 Chapter 4 Cautions
Preface

How to use this guide

Purpose of This Guide

Samples including plant, animal and microbes (fungus) were grinded with the motor-driven tissue grinder G50. The efficiency of G50 grinding each samples together with the concentration and the purity of the RNA extracted were compared with those obtained with the mortar and the grinding machine, through which the application range of G50 was verified and its advantages was more obviously illuminated so as to serve the users better.

Audience

This guide is intended for novice G50 users who wants to get better results.
Chapter 1 Plant tissue

Efficiency of G50 for different plant tissues’ grinding

<table>
<thead>
<tr>
<th>Device</th>
<th>Motor tissue grinder G50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Rice root</td>
</tr>
<tr>
<td>Amount (g)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Method
1. Cut the samples into pieces and put them into a 1.5mL centrifuge tube;
2. Open the tube lid and sink it into the liquid nitrogen, keeping it submerged for >5 secs. Then place the tube on the tube rack;
3. Keep grinding the samples for no more than 10secs using the pestle precooled with the liquid nitrogen before the liquid volatilizes. Repeat this grinding step (less than 10 secs) for several times.

<table>
<thead>
<tr>
<th>Grinding speed (krpm)</th>
<th>3~5</th>
<th>&gt;10</th>
<th>5~8</th>
<th>5~8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grinding time (sec)</td>
<td>5</td>
<td>Keep grinding for several times</td>
<td>$5 \times (1-3 \text{ times})$</td>
<td>$5 \times (1-3 \text{ times})$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Efficiency</th>
<th>Grinded into powder quite easily</th>
<th>Only a little was grinded into particles</th>
<th>Grinded into powder and particles easily</th>
<th>Grinded into powder and particles easily</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influence Factors</td>
<td>Sample amount</td>
<td>Sample amount; different parts</td>
<td>Sample amount; leaf’s age</td>
<td>Sample amount; leaf’s age</td>
</tr>
</tbody>
</table>

Before

![Rice root](image1.jpg) ![Rice stem](image2.jpg) ![Rice leaf](image3.jpg)

After

![Rice root](image4.jpg) ![Rice stem](image5.jpg) ![Rice leaf](image6.jpg)
**Conclusion:**

* Low speed is recommended to break the samples into pieces and make them gathered at the bottom, then high speed is used to grind thoroughly.
* It is advised to fix the centrifuge tubes on the rack during the grinding. Move the pestle up softly up and down to homogenize the samples more uniformly.
* Plastic pestles are recommended to use in the plant tissue grinding experiments.
* Avoid keeping continual grinding for more than 10 secs in that heat produced may affect the results.
* Rice roots could be grinded most easily while its stem and seeds would be tough to grind. Both the rice leaf and bamboo leaf are recommended to be grinded with a little amount, or it would be grinded unthoroughly.

According to the results, plant tissues grinded with G50 will have good performance. (Stem sample would be a little hard to grind)
Efficiency comparison of different methods for grinding the rice leaves

<table>
<thead>
<tr>
<th>Device</th>
<th>Mortar</th>
<th>G50</th>
<th>Glass pestle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Rice leaf (~0.1g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Cut the leaves into pieces and sink them into the mortar filled with liquid nitrogen. Grind with the rod before the liquid nitrogen volatilizes and add in the liquid nitrogen constantly till the samples are broken into powder.</td>
<td>The same as in experiment 1.</td>
<td>Automatic grinding replaced with manual operation.</td>
</tr>
<tr>
<td>Grinding time (sec)</td>
<td>5 × (3 times) liquid nitrogen is added constantly</td>
<td>5 × (1-3 times)</td>
<td>5 × 3 times</td>
</tr>
<tr>
<td>Efficiency</td>
<td>Grinded into powder thoroughly</td>
<td>Grinded into powder or particles</td>
<td>Grinded into powder or particles</td>
</tr>
<tr>
<td>Advantages</td>
<td>Large amount of samples can be grinded thoroughly</td>
<td>Time and energy saving; contamination avoided</td>
<td>Little sample loss</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>Operating inconvenience, easy contamination and large sample loss</td>
<td>Slight sample loss and a little bit weaker than the mortar</td>
<td>Hard work required and the tube is very likely to be broken during the operation</td>
</tr>
</tbody>
</table>

Conclusion:
The mortar is inconvenient to be used and brings with easy contamination and large sample loss. The glass pestle is hard to operate when large amount of sample is needed and the risk of cold injury due to the liquid nitrogen is high. On the contrary, G50 is more time and energy saving with little contamination and loss, especially suitable to the nucleic acid extraction from precious scarce samples.
Quality comparison of the RNA extracted from the rice leaves grinded through different methods

<table>
<thead>
<tr>
<th>Device</th>
<th>Instruments and reagents required for a common RNA extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Rice leaf grinded with the mortar</td>
</tr>
<tr>
<td></td>
<td>Rice leaf grinded with the G50</td>
</tr>
<tr>
<td></td>
<td>Rice leaf grinded with the glass pestle</td>
</tr>
<tr>
<td>Detection method</td>
<td>Agarose gel electrophoresis</td>
</tr>
<tr>
<td>RNA concentration (μg/μL)</td>
<td>380.6</td>
</tr>
<tr>
<td></td>
<td>537.8</td>
</tr>
<tr>
<td></td>
<td>317.4</td>
</tr>
<tr>
<td>OD260/280</td>
<td>2.14</td>
</tr>
<tr>
<td></td>
<td>2.15</td>
</tr>
<tr>
<td></td>
<td>2.14</td>
</tr>
</tbody>
</table>

Electrophoresis result

Conclusion:

The concentration of RNA extracted from the samples grinded with the mortar is smaller than that of the G50 due to a large sample loss.
## Chapter 2 Animal tissue

### Efficiency of G50 for different animal tissues’ grinding

<table>
<thead>
<tr>
<th>Tissue resource</th>
<th>Grinding speed (krpm)</th>
<th>Efficiency</th>
<th>Method</th>
<th>Influence factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>10</td>
<td></td>
<td>1. Add no more than 400 μL lysis buffer or Trizol into the 1.5 mL centrifuge tube; 2. Break the samples and put one patch into the tube immediately; 3. Break the samples into pieces under a low speed to gather them at the bottom and grind under a high speed. 4. Move the pestle up softly up and down to homogenize the samples more uniformly. Avoid keeping grinding for more than 10 secs for the heat produced may affect the results. Repeat the grinding step twice or three times. Keep holding the tube and the grinder with each hand during the operation. Do not grind when the tube is fixed on the rack. Metal pestles are recommended.</td>
<td>The type of the tissue, amount, freshness</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear &amp; skin</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marrow</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Large intestine</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small intestine</td>
<td>8-10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testis</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonsil</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph gland</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Longissmus</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood vessel</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: Little grinded □ Small block residue □ Batt-like structure □ Seriflux □
Conclusion:

* The volume of the liquid should be no more than 400 μL in case that the droplet splashes out to cause the sample loss. The sample tissues could be broken and homogenized and then the reagent could be added to the required volume (e.g. >400 μL).
* Avoid keeping grinding for more than 10 secs in case that the heat produced damages the nucleic acid. Repeat grinding twice or three times.
* Keep holding the tube and the grinder with each hand during the operation. Do not grind when the tube is fixed on the rack. Metal pestles are recommended.
* Tissues from the liver, spleen, brain, marrow, large intestine could be grinded easily while those tough tissues such as the skin, ear and blood vessel is a bit tough to grind. The grinding efficiency of the skeletal muscle depends on its freshness. The more fresh, the harder it is to be grinded.

According to the results, G50 widely applies to the animal tissues’ grinding.
### Efficiency comparison of different grinding methods for the longissimus muscle

<table>
<thead>
<tr>
<th>Device</th>
<th>Mortar</th>
<th>G50</th>
<th>Glass pestle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Materials</strong></td>
<td>Longissimus muscle (0.3g)</td>
<td>The same as in experiment 1.</td>
<td>Automatic grinding replaced with manual operation.</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>The same as in experiment 1.</td>
<td>The same as in experiment 1.</td>
<td>Automatic grinding replaced with manual operation.</td>
</tr>
<tr>
<td><strong>Grinding time (sec)</strong></td>
<td>5 × (3 times) liquid nitrogen is added constantly</td>
<td>5 × (2-3 times)</td>
<td>60</td>
</tr>
<tr>
<td><strong>Efficiency</strong></td>
<td>Grinded into powder thoroughly</td>
<td>Grinded into batt-like seriflux</td>
<td>Little was broken.</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Large amount of samples can be grinded thoroughly</td>
<td>Time and energy saving; contamination avoided</td>
<td>Large amount of samples can be treated at the same time.</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Operating inconvenience, easy contamination and large sample loss</td>
<td>The grinder is hard to hold steady when working at a high speed. The droplets are easy to splash out</td>
<td>Samples are hard to be broken thoroughly. Tissues from the muscles and the skin couldn’t be grinded</td>
</tr>
</tbody>
</table>

**Conclusion:**

The advantages of G50 is obviously superior to those of the grinding machine and the mortar. Keep in mind avoiding splashing out the droplets.
Chapter 3 Microbes

Effectiveness comparison of different methods for grinding the fungus hypha

<table>
<thead>
<tr>
<th>Device</th>
<th>Mortar</th>
<th>G50</th>
<th>Grinding machine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Aspergillus niger(0.02g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td></td>
<td>1 Cut the samples into pieces and put them into a 1.5mL centrifuge tube; Open the tube lid and sink it into the liquid nitrogen, keeping it submerged for &gt;5 secs. Then place it on the tube rack; Keep grinding the samples for no more than 10secs using the pestle cooled with the liquid nitrogen previously before the liquid volatilizes. Move the pestle up softly up and down to homogenize the samples more uniformly.</td>
<td>2 Add no more than 400 μL lysis buffer or Trizol into the 1.5 mL centrifuge tube; gather the hypha at the bottom under a low speed and under a high one. Move the pestle up and down and keep grinding for &lt;10s. grindcontaining the samples and start the machine under the highest speed after the samples are precooled with the liquid nitrogen. Keep grinding for 1 min.</td>
</tr>
<tr>
<td>Grinding time (sec)</td>
<td>5 × (3 times)</td>
<td>&lt;10krpm, 5 × 5 times)</td>
<td>10krpm, 5 × (2-3 times)</td>
</tr>
<tr>
<td>Efficiency</td>
<td>Grinded into powder thoroughly</td>
<td>Little was broken</td>
<td>Grinded into batt-like seriflux.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grinded into powder</td>
<td></td>
</tr>
</tbody>
</table>

11
**Conclusion:**

* The hypha was as hard as the rocks after cooled by the liquid nitrogen. G50 is not recommended in such case. The Trizol or other lysis buffer should be added to grind directly.
* Grinding with the mortar or the grinding machine caused large sample loss due to that a large part of samples were adhered to the inwall and the steel ball. On the contrary, the samples were grinded in the buffer and was hardly adhered to the machine, thus causing little loss.

**Quality comparison of the RNA extracted from the fungus hypha grinded through different methods**

<table>
<thead>
<tr>
<th>Device</th>
<th>Instruments and reagents required for a common RNA extract</th>
<th>Detection method</th>
<th>RNA concentration (μg/μL)</th>
<th>OD260/280</th>
<th>Electrophoresis result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials (0.01g)</td>
<td>Hypha grinded with the mortar</td>
<td>Agarose gel electrophoresis</td>
<td>378.4</td>
<td>2.13</td>
<td><img src="image1" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>Hypha grinded with G50 (in the 2nd way)</td>
<td></td>
<td>638.5</td>
<td>2.13</td>
<td><img src="image2" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td>Hypha grinded with the grinding machine</td>
<td></td>
<td>412.6</td>
<td>2.14</td>
<td><img src="image3" alt="Image" /></td>
</tr>
</tbody>
</table>

**Conclusion:**

Given that large sample loss would be caused when grinding with the mortar and the grinding machine, which results in a smaller concentration of RNA extracted than that of the grinder, G50 is more convenient than the mortar and applies well to the nucleic acid extraction from small amount of samples such as the fungus hyph.
## Chapter 4 Cautions

### Cautions for use

<table>
<thead>
<tr>
<th>Pestle</th>
<th>1) Cleaning: Clean the pestles timely with the alcohol, detergent or ultrasonic wave and autoclave after use to prevent rust and residues.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2) Installation: Press the pestle a little bit deeper after inserting into the rubber ring.</td>
</tr>
<tr>
<td></td>
<td>3) Replacement: Replace the plastic pestle regularly in case that the pestles are distorted for frequent use.</td>
</tr>
<tr>
<td>Operation</td>
<td>1) Avoid starting the machine outside the tube. Stop it when moving out.</td>
</tr>
<tr>
<td></td>
<td>2) Hold the machine tightly to prevent slipping.</td>
</tr>
<tr>
<td></td>
<td>3) The volume of the liquid should be no more than 400 μL in case that the droplet splashes out. The samples could be broken and homogenized and then the reagent could be added to the required volume (eg, &gt;400 μL).</td>
</tr>
<tr>
<td></td>
<td>4) Avoid keeping grinding for more than 10 secs for the heat produced may affect the results.</td>
</tr>
<tr>
<td></td>
<td>5) Avoid strong acid or alkaline and most of the organic solvents. Salt solutions also slowly corrode metal parts and therefore, should be avoided as well.</td>
</tr>
<tr>
<td></td>
<td>6) The grinder is not water resistant. Absolutely no immersion into water.</td>
</tr>
</tbody>
</table>
To the readers:

This guide was written according to the experiment results from our technicians for the customers’ reference only. We appreciate any comments and opinions on any possible deficiencies and pls feel free to contact us with the following information.

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