

# Homogenizer

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

The Invitrogen™ Homogenizer is a cartridge fitted with a specialized membrane that utilizes centrifugal force to reduce the viscosity of a lysate. It provides convenient, consistent homogenization of cells or tissue prior to nucleic acid isolation and is particularly effective for homogenizing plant tissue.

The Homogenizer is recommended for use with the Ambion™ PureLink™ RNA Mini Kit to isolate total RNA.

## Sample types

The Homogenizer is recommend for the following sample types.

- $<1 \times 10^7$  tissue culture cells (animal or plant; monolayer or suspension)
- $<100$  mg of animal tissue
- $<250$  mg of plant tissue
- $<5 \times 10^8$  yeast cells
- $<1 \times 10^9$  bacteria cells

## Homogenize animal or plant cells

Use the following protocols to process  $<1 \times 10^7$  cells in monolayer or suspension. For larger samples, use a rotor-stator homogenizer.

### Monolayer cells

1. Remove growth medium.
2. Add  $<0.6$  mL of RNA Lysis Solution evenly over the entire surface of the culture dish.
3. Pipet the buffer over the surface until the cells appear lysed.
4. Transfer the lysate to the Homogenizer.
5. Centrifuge at  $12,000 \times g$  for 2 minutes at  $25^\circ\text{C}$ .
6. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

### Suspension cells

1. Pellet cells in a 1.5 mL tube at  $2000 \times g$  for 5 minutes at  $4^\circ\text{C}$ .
2. Decant growth medium.
3. Add  $<0.6$  mL of RNA Lysis Solution to the cell pellet and vortex to disrupt the cells.
4. Transfer the lysate to the Homogenizer.
5. Centrifuge at  $12,000 \times g$  for 2 minutes at  $25^\circ\text{C}$ .
6. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Homogenize animal tissue

Use the following protocol to process up to 100 mg of tissue. For samples  $>100$  mg, use a rotor-stator homogenizer.

### Prepare tissue sample

Process tissue samples of  $>10$  mg according to the following table:

| Sample type                           | Action                          |
|---------------------------------------|---------------------------------|
| Fresh tissue                          | Mince tissue quickly            |
| Frozen tissue or fresh fibrous tissue | Grind tissue in liquid nitrogen |

### Samples with $<25$ mg of tissue

1. Place the sample in a 2-mL round-bottom tube on ice.
2. Add 0.3–0.6 mL RNA Lysis Solution to the sample and disrupt with up and down strokes of a pestle.
3. Transfer the lysate to the Homogenizer.
4. Centrifuge at  $12,000 \times g$  for 2 minutes at  $25^\circ\text{C}$ .
5. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Samples with 25–60 mg of tissue

1. Place the sample in a 2-mL round-bottom tube on ice.
2. Add 0.3–0.6 mL RNA Lysis Solution to the sample and disrupt with up and down strokes of a pestle.
3. Transfer the lysate to the Homogenizer.
4. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
5. Pass the homogenate through the Homogenizer a second time using the same collection tube.
6. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
7. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Samples with 60–100 mg of tissue

1. Place the sample in a 2-mL round-bottom tube on ice.
2. Add 0.6 mL RNA Lysis Solution to the sample and disrupt with up and down strokes of a pestle.
3. Add another 0.6 mL RNA Lysis Solution to the sample and mix.
4. Transfer 0.6 mL of the lysate to each of two Homogenizers.
5. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
6. Pass the homogenate through the Homogenizer a second time using the same collection tube.
7. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
8. Remove the Homogenizers from the collection tubes.
9. Combine the contents of the two collection tubes, and perform nucleic acid isolation.

## Homogenize plant tissue

Use the following protocol to process up to 250 mg of plant tissue.

### Prepare plant sample

Process samples according to the following table:

| Sample type                           | Action  |
|---------------------------------------|---|
| Fresh non-fibrous soft tissue         | Cut up tissue quickly, and transfer to a rotor-stator homogenizer |
| Frozen tissue or fresh fibrous tissue | Grind tissue in liquid nitrogen and keep tissue powder frozen     |

## Homogenize ground, frozen plant tissue

1. Add 0.5–1 mL of RNA Lysis Solution to the sample, and vortex or tap the tube to disperse the tissue powder.
2. Incubate for 3 minutes.
3. Pour 0.5 mL of lysate into the Homogenizer, or 0.5 mL of lysate into each of two Homogenizers if processing 1 mL of sample.
4. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
5. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Homogenize non-fibrous, soft, fresh plant tissue

1. Add 0.5–1 mL of RNA Lysis Solution to the sample.
2. Homogenize with a rotor-stator homogenizer for 1 minute.
3. Pour 0.5 mL of lysate into the Homogenizer, or 0.5 mL of lysate into each of two Homogenizers if processing 1 mL of sample.
4. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
5. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Homogenize yeast cells

Use the following protocol to process up to  $5 \times 10^8$  yeast cells.

1. Crush approximately 10 g of dry ice in a mortar (5-cm inner diameter) to a powder.
2. Resuspend up to  $5 \times 10^8$  yeast cells in 500  $\mu$ L of RNA Lysis Solution.
3. Add the suspension drop-wise onto the crushed dry ice. Grind the mixture with a pestle until the dry ice has evaporated and the paste begins to melt.
4. Transfer the liquid to the Homogenizer.
5. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
6. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Homogenize bacterial cells

Use the following protocol to process up to  $1 \times 10^9$  log phase bacterial cells.

1. Lyse up to  $1 \times 10^9$  pelleted bacterial cells in 100  $\mu$ L of 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA containing 1 mg lysozyme. Vortex the pellet until resuspended.
2. Add 0.5 mL of 10% SDS solution and mix well. Incubate at room temperature for 5 minutes.
3. Add 350  $\mu$ L of RNA Lysis Solution and mix thoroughly.
4. Transfer all of the liquid to the Homogenizer.
5. Centrifuge at  $12,000 \times g$  for 2 minutes at 25°C.
6. Remove the Homogenizer from the collection tube, and perform nucleic acid isolation.

## Troubleshooting

| Observation   | Possible cause   | Recommended action   |
|---|--|--|
| Lysate does not pass into the collection tube after centrifugation. | Homogenizer may be clogged. Highly viscous lysates (e.g., calf thymus) can cause clogging. | Use a rotor-stator homogenizer to process the sample.  |
|   | Homogenizer may be clogged due to too much sample being added.                             | Reduce the amount of sample added to the Homogenizer (See "Sample types" on page 1 for recommended amounts). |

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