Imagen® ImaZol

For isolation of total RNA (large and small RNA) and for isolation of large RNA (> 200 base) and small RNA (< 200 base) in two separate fractions from a variety of sample materials.

Precautions

I. Handling requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and reagents to be supplied by user

- 75% ethanol
- 100% isopropanol
- 70% isopropanol
- RNase-free Water
- 1.5 mL or 2mL microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortex mixer
- Microcentrifuge (with rotor for 1.5 mL or 2mL tubes) may be required for some samples
- 4 – bromoanisole(optional)

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- When handling RNA and reagent, wear gloves to avoid contact with skin. Because skin is a source of RNase.

Kit Contents

ImaZol

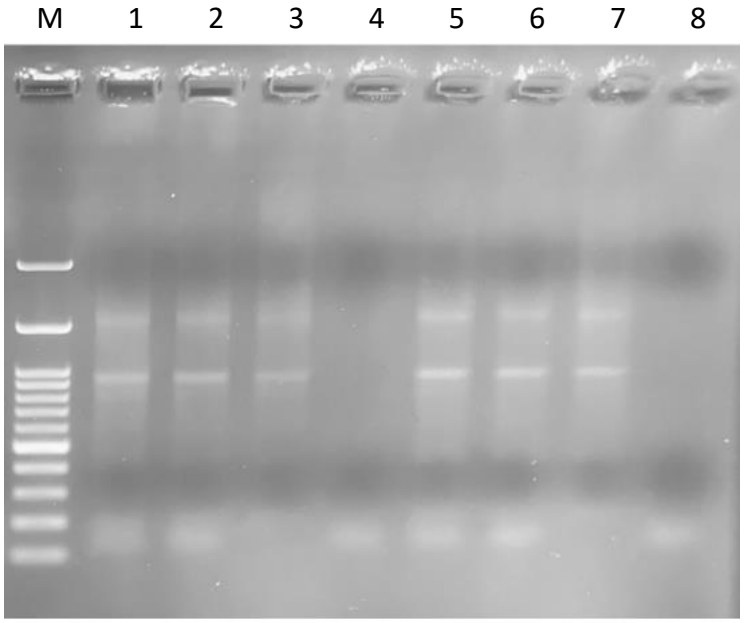
Storage and Stability:

This kit should be stored at room temperature.

Description

ImaZol is designed for the isolation of total RNA in a single fraction, large RNA and small RNA in a separate fraction from a variety of samples including tissue, cells, bacteria, yeast, and other materials. The protocol uses ImaZol reagent based on guanidium salt and phenol to lyse and homogenize samples. Contaminants(e.g. DNA, protein and polysaccharide) are precipitated by adding water then centrifuging to remove. Via adding ethanol and isopropanol to isolate large RNA and small RNA separately. After washing off the contaminants, the purified RNA is eluted by RNase-free water. Purified RNA is ready for qRT-PCR, northern blotting, RNase protection assay, primer extension, microarrays, cDNA library construction, and other application.

ImaZol Test Data



Total RNA, large RNA, and small RNA from 10 mg Fish (*Oreochromis mossambicus*) liver were extracted using ImaZol and competitor Zol. 5 µl from 100 µl eluates of purified total RNA, large RNA and small RNA were analyzed by electrophoresis on a 1.5 % agarose gel.

M = 1 Kb DNA Ladder
1 = Competitor Zol Reagent, total RNA with phase separation
2 = Competitor Zol Reagent, total RNA
3 = Competitor Zol Reagent, large RNA
4 = Competitor Zol Reagent, small RNA
5 = ImaZol, total RNA with phase separation
6 = ImaZol, total RNA
7 = ImaZol, large RNA
8 = ImaZol, small RNA

Preparation before using

A RNase-free environment is a key point for successful RNA isolation, please avoid RNase contamination following wear gloves and mask, keep a single area to operate experiment, use sterile RNase-free centrifugation tube, and keep reagent sealed when not in use. And read all steps from protocol procedure carefully before operating.

Isolation of total RNA protocol procedure

1. Sample preparation and homogenization.

   Tissue

   Homogenize tissue samples with mechanical disruption using up to 50 mg of tissue per 500 µl ImaZol.

   Note: if the tissue has high DNA content (e.g., spleen tissue), use 500 µl of reagent per 25 mg of tissue.

   Cells

   Suspension cells: isolate cells by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 5 $\times 10^6$ cells.

   Monolayer cells: after removing culture medium, use at least 1 mL of ImaZol per 10 cm^2 of glass culture plate surface area. Then ensure complete lysis by pipetting several times.

   Note: Do not wash cells prior to addition of ImaZol. Washing cells might contribute to RNA degradation.

   Note: Insufficient volume of the reagent will lead to DNA contamination of the isolated RNA.

   Bacteria

   Isolate bacteria by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 1 $\times 10^9$ cells.

   Note: if there are more bacteria than 1 $\times 10^9$ cells, may make DNA contamination worse. Please increase the volume of ImaZol or refer to protocol step 3 (phase separation).

   Yeast

   Isolate yeast by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 1 $\times 10^8$ cells.

   Note: if there are more yeast than 1 $\times 10^8$ cells, may make DNA contamination worse. Please increase the volume of ImaZol or refer to protocol step 3 (phase separation).

2. Precipitate contaminants (e.g., DNA, protein and polysaccharide):
   - Add 200 µl RNase-free water per 500 µl ImaZol to the lysate. Vortex the sample vigorously for 15 seconds. Incubate at room temperature for 10 minutes.
   - Centrifuge samples for 15 minutes at 12000 x g at room temperature.
     Note: Centrifugation separates the mixture into a semisolid pellet (containing DNA, proteins and polysaccharides) and an upper supernatant (containing RNA).
   - Transfer 500 µl supernatant to a new tube. Leave the layer of the supernatant above the DNA /protein pellet.

3. Phase separation (**optional**):

   Note: The basic protocol for total RNA isolation can be complemented by an optional phase separation. This is useful for samples with high DNA content and/or extracellular material.
   - Add 2.5 µl (0.5 % of supernatant volume) 100% 4-bromoanisole to 500 µl transferred supernatant. Vortex well for 15 seconds and incubate for 5 minutes at room temperature.
     Note: Do not substitute bromochloropropane or chloroform for 4-bromoanisole.
   - Centrifuge for 10 minutes at 12,000 x g at room temperature.
   - Transfer the supernatant to a new microcentrifuge tube, which contains the RNA.
     Note: residual DNA, proteins and polysaccharides are accumulated in the organic phase at the bottom of the microcentrifuge tubes.

4. Precipitate total RNA:
   - Transfer RNA containing supernatant from step 2 or 3 into a new microcentrifuge tube.
   - Add 500 µl of 100% isopropanol per 500 µl supernatant and mix well in order to precipitate RNA.
   - Incubate at room temperature for 10 minutes.
   - Centrifuge for 10 minutes at 12000 x g.

Note: Even if no deposits are clearly visible, proceed to the next step.
- Remove and discard the supernatant.

5. Wash RNA pellet once with 700 µl of 75% ethanol.
   - Inverting 10 times.
   - Centrifuge for 3 minutes at 8000 x g.
   - Remove ethanol from RNA pellet by pipetting.
   - Note: Do not dry the pellet, which may lead to a decrease of solubility.
6. Dissolve RNA with RNase-free water to obtain a concentration of 1-2 µg/µL.
   - Vortex 2 minutes at room temperature for efficient solubilization.
     Note: Confirm whether the precipitate is completely dissolved, and increase the vortexing time if necessary.

Isolation of large and small RNA in two separate fraction protocol procedure

1. Sample preparation and homogenization.
   Tissue
   Homogenize tissue samples with mechanical disruption using up to 50 mg of tissue per 500 µl ImaZol.
   Note: if the tissue has high DNA content (e.g., spleen tissue), use 500 µl of reagent per 25 mg of tissue.
   Cells
   Suspension cells: isolate cells by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 5 $\times 10^6$ cells.
   Monolayer cells: after removing culture medium, use at least 1 mL of ImaZol per 10 cm^2 of glass culture plate surface area. Then ensure complete lysis by pipetting several times.
   Note: Do not wash cells prior to addition of ImaZol. Washing cells might contribute to RNA degradation.
   Note: Insufficient volume of the reagent will lead to DNA contamination of the isolated RNA.
   Bacteria
   Isolate bacteria by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 1 $\times 10^9$ cells.
   Note: if there are more bacteria than 1 $\times 10^9$ cells, may have more residual DNA. So increase the volume of ImaZol.
   Yeast
   Isolate bacteria by centrifugation and lyse directly by the addition of ImaZol to vortex vigorously for 15 seconds. And 500 µl of reagent is sufficient to lyse 1 $\times 10^8$ cells.
   Note: if there are more bacteria than 1 $\times 10^8$ cells, may have more residual DNA. So, increase the volume of ImaZol or refer to protocol step 3 (phase separation).
2. Precipitate contaminants (e.g., DNA, protein and polysaccharide):
   - Add 200 µl RNase-free water per 500 µl ImaZol to the lysate. Vortex the sample vigorously for 15 seconds. Incubate at room temperature for 10 minutes.
   - Centrifuge samples for 15 minutes at 12000 x g at room temperature.
     Note: Centrifugation separates the mixture into a semisolid pellet (containing DNA, proteins and polysaccharides) and an upper supernatant (containing RNA).
   - Transfer 500 µl supernatant to a new tube. Leave the layer of the supernatant above the DNA /protein pellet.
3. Precipitate large RNA:
   - Transfer RNA containing supernatant from step 2 into a new microcentrifuge tube.
   - Add 200 µl 75% ethanol in supernatant and mix well in order to precipitate RNA.
   - Incubate at room temperature for 10 minutes.

- Centrifuge for 10 minutes at 12000 x g.
- Note: Even if no deposits are clearly visible, proceed to the next step.
- Transfer the supernatant containing the small RNA to a new microcentrifuge tube by pipetting.
  Note: the supernatant containing small RNA can be stored at 4°C for up to 24 hours or at -20°C to -80°C for up to one year.

4. Precipitate small RNA:
   - Add 560 µl of 100% isopropanol (~0.8 volume) in supernatant obtained from precipitate RNA (step3) and mix well in order to precipitate RNA.
   - Incubate at 4°C for 30 minutes.
   - Centrifuge for 15 minutes at 12000 x g at room temperature.
     Note: Even if no deposits are clearly visible, proceed to the next step.
   - Remove and discard the supernatant by pipetting.
5. Wash RNA pellet:
   Large RNA
   - add 700 µl 75% ethanol to wash the pellet.
   - Inverting 10 times.
   - Centrifuge for 3 minutes at 8000 x g.
   - Remove ethanol from RNA pellet by pipetting.
     Note: Do not dry the pellet, which may lead to a decrease of solubility.
     Note: only wash once.

   Small RNA
   - Add 700 µl 70% isopropanol to wash the pellet.
   - Inverting 10 times.
   - Centrifuge for 3 minutes at 8000 x g.
   - Remove ethanol from RNA pellet by pipetting.
     Note: Do not dry the pellet, which may lead to a decrease of solubility.
     Note: only wash once.

6. Dissolve RNA with RNase-free water to obtain a concentration of 1-2 µg/µL.
   - Vortex 2 minutes at room temperature for efficient solubilization.
     Note: Confirm whether the precipitate is completely dissolved, and increase the vortexing time if necessary.

Troubleshooting

Problem	Possible Reasons / Solution
Low RNA Yield	➢ Incomplete homogenization or lysis of samples. / Testing more efficient condition to improve homogenization. ➢ The RNA pellet may not been completely dissolved. / Increase the vortexing time or increase the volume of RNase-free water for dissolving of RNA.
RNA Degradation	➢ Inappropriate handling of starting material. / Harvested sample immediately stabilized. ➢ RNase contamination / Avoid RNase contamination by always wear gloves & mask and treat all the equipment with RNaseOUT.
A_{260}/A_{280} ratio < 1.6	➢ Insufficient starting material. / Increase the amount of sample. ➢ The volume of ImaZol used for homogenization was insufficient. / Increase the volume of ImaZol. ➢ The RNA pellet may be only partly solubilized. / Increase the vortexing time or increase the volume of RNase-free water for dissolving of RNA. ➢ Contamination of proteoglycan or polysaccharide. / Implement phase separation as described in total RNA protocol step 3.
Contamination with DNA	➢ The volume of ImaZol used for homogenization was insufficient. / Increase the volume of ImaZol. ➢ Organic solvents, alkaline solution, strong buffer, or salt contamination. / Precipitate contaminants (protocol step 2) can be improved by increasing incubation time to 15 minutes and increasing speed of centrifugation at 16000 x g. ➢ ImaZol can efficiently remove DNA when processing samples according to the standard protocol, the residual among of DNA is minimal. If processing sample with high levels of DNA, it may be hard to remove all DNA. / RNase-free, high quality, commercial DNase can facilitate to remove contamination DNA.