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# Simzol Instructions

## Composition

| Cat. No. | 5302006 | 5302100 | 5302200 |
|---|---|---|---|
| Simzol | 6 ml | 100 ml | 200 ml |
| Instructions | 1 | 1 | 1 |

## Storage

If the reagent is stored at room temperature (15~25℃), it can keep the performance for 1 year without obvious change, and if stored at 2~8℃, the validity period can be extended to more than 3 years.
When the room temperature is ≥25℃, the product must be stored at 2~8℃. Products stored at 2~8℃ must be restored to room temperature (≥15℃) before use, see "Preparation before use" for details.

## Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

## Introduction

Simzol is suitable for extract total RNA from various samples such as cells, tissues and fluids of human or animal origin, plants, yeast, bacteria, viral materials, and more. In homogenized or samples lysate, Simzol preserves RNA integrity while disrupting cells and lysing cellular components. Simzol does not need to add chloroform, just add RNase-free water and mix and centrifuge, impurities such as DNA, polysaccharides and proteins can be precipitated and removed, RNA is present in the supernatant, and miRNA and large RNA can be isolated by adding ethanol and isopropanol, respectively.
Total RNA extracted from Simzol can be used for Northern blot analysis, dot blot, poly(A)+ selection, in vitro translation, RNase protection analysis, and molecular cloning. Simzol removes most of the DNA from the sample, but not completely, so DNase I (Simgen Cat. No. 8003050) to process the isolated RNA or select a cDNA first-strand synthesis kit (Simgen Cat. No. 7306100) is recommended.

## Equipment And Reagents to Be Supplied by Users

1. RNase-free water or DEPC treated water, isopropanol, 75% ethanol (prepared with DEPC treated water).
2. RNase-free1.5 ml centrifuge tubes, pipettes and tips.
3. Disposable gloves and protective equipment and tissues.
4. Microcentrifuge (s) (with rotor for 1.5 ml and 2 ml centrifuge tubes).
5. Liquid nitrogen, mortar and a homogenizer (animal tissue) may be required.
6. PBS solution, 70% isopropanol (prepared with DEPC-treated water) may be required.
7. RNase-free labs.

## Preparation before use

1. Since the cloud point of Simzol is around 10℃, Simzol stored at 2~8℃ will become turbid and eventually form a split phase. Before use, it must be restored to room temperature (incubate to 20℃) and mixed to form a transparent and uniform solution before use.
2. Note: Simzol contains irritating compounds that can corrode the skin and must be handled with gloves and goggles.
3. If the centrifuge has a refrigeration function, set the temperature to 25℃.
4. RNase-free water treatment method: Deionized water was added to sterilizable glass containers, diethyl pyrophosphate (DEPC) was added to a final concentration of 0.1% (v/v), and left at 37℃ overnight, 121℃, sterilized for 20 min.

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# Protocol For Total RNA Extraction

**This protocol is designed for the total RNA extraction by 500 µl Simzol, and if RNA is being extracted from more tissues or cells, the amount of Simzol, isopropanol, 75% ethanol, etc., must be increased proportionally. If RNA is extracted from trace tissues or cells (1~10 mg tissue or $10^2$~$10^4$ cells), add 5~10 µg Carrier RNA (Simgen Cat. No. 4003101) to the isopropanol adding step. The presence of Carrier RNA does not affect RT-PCR.**

1. **Treatment for different samples sources:**

   **Animal or plant tissues/plant cells/yeasts/bacteria:**

   Grind about 100~200 mg of tissue to powder form with liquid nitrogen in a mortar, weigh 25~100 mg of tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 500 µl Simzol, close the lid, shake vigorously to mix well, and enter the operation of step 3.

   * Try to add Simzol before the tissue powder thaws to reduce the degradation of RNA by endogenous RNases in tissues.

   * Animal tissues can be lysed with a homogenizer or other mechanical disruption method with the addition of 500 µl Simzol.

   **Cultured animal cells:**

   **Adherent cells:** Add 500 µl Simzol per 10 $cm^2$ of cultured cells (e.g., a 3.5 cm diameter cell culture dish, discard the medium, add 500 µl Simzol directly), pipette the cells several times with the tip to lyse the cells, transfer the homogenate into a 1.5 ml centrifuge tube, and proceed to step 3.

   **Suspension cultured cells:** Centrifuge 5~10×$10^6$ cells with a 1.5 ml centrifuge tube, add 200 µl PBS solution, vortexing until the cells are all suspended, add 500 µl Simzol, directly pipette the cells several times with the tip to lyse the cells, note that there is no need to add 200 µl RNase-free water, and directly proceed to step 4.

   **Liquid samples:**

   Add 500 µl Simzol per 200 µl liquid sample (e.g., blood, plasma, etc.), close the lid, and shake vigorously to mix. When the sample volume is less than 200 µl, make up to 200 µl with RNase-free water, and add 500 µl Simzol to mix well. Note: There is no need to add 200 µl RNase-free water and directly proceed to step 4.

2. ***Optional step:*** **If the sample contains more lipids, centrifuge at 12,000×g for 5 min. After centrifugation, a fat layer will appear on top, transfer the lower layer of clear solution into a new 1.5 ml centrifuge tube with a tip to proceed to the next step.**
3. **Add 200 µl RNase-free water. Close the lid, shake vigorously for 15 sec.**
4. **Centrifuge at 12,000 ×g for 15 min and transfer 500 µl supernatant into a new RNase-free 1.5 ml centrifuge tube.**
5. **Add 500 µl isopropanol, mix well, centrifuge at 12,000×g for 10 min, and discard the supernatant.**
6. **Add 1 ml 75% ethanol, gently invert the tube 4~6 times, centrifuge at 8,000×g for 5 min, and discard the supernatant.**

   * If a large amount of RNA precipitation is observed, repeat step 6 once to reduce the residual salts in the RNA.

7. **Close the lid and spin down. Discard the residual ethanol with a 200 µl tip, leaving the white RNA pellet at the bottom and wall the tube. There is no need to dry the RNA.**

   * Note: When RNA is extracted from some samples, the RNA does not form white spots at the bottom of the tube, but rather adsorbs to the wall the tube as a uniform mist-like precipitate. Observe carefully and pay special attention to dissolving the RNA by adding RNase-free water to the appropriate location on the tube wall during step 8.

8. **Add 50~100 µl RNase-free water to dissolve the RNA. Store the RNA below -70°C for later use.**

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# Protocol For miRNA and Large Fragments RNA Separate Extraction

**This protocol is designed for RNA extraction by 500 µl Simzol, and if RNA is being extracted from more tissues or cells, the amount of Simzol, 75% ethanol, isopropanol, and 70% isopropanol must be increased proportionally.**

1. **Treatment for different samples sources:**

   **Animal or plant tissues/plant cells/yeasts/bacteria:**

   Grind about 100~200 mg of tissue to powder form with liquid nitrogen in a mortar, weigh 25~100 mg of tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 500 µl Simzol, close the lid, shake vigorously to mix well, and enter the operation of step 3.

   * Try to add Simzol before the tissue powder thaws to reduce the degradation of RNA by endogenous RNases in tissues.

   * Animal tissues can be lysed with a homogenizer or other mechanical disruption method with the addition of 500 µl Simzol.

   **Cultured animal cells:**

   **Adherent cells:** Add 500 µl Simzol per 10 $cm^2$ of cultured cells (e.g., a 3.5 cm diameter cell culture dish, discard the medium, add 500 µl Simzol directly), pipette the cells several times with the tip to lyse the cells, transfer the homogenate into a 1.5 ml centrifuge tube, and proceed to step 3.

   **Suspension cultured cells:** Centrifuge 5~10×$10^6$ cells with a 1.5 ml centrifuge tube, add 200 µl PBS solution, vortexing until the cells are all suspended, add 500 µl Simzol, directly pipette the cells several times with the tip to lyse the cells, note that there is no need to add 200 µl RNase-free water, and directly proceed to step 4.

   **Liquid samples:**

   Add 500 µl Simzol per 200 µl liquid sample (e.g., blood, plasma, etc.), close the lid, and shake vigorously to mix. When the sample volume is less than 200 µl, make up to 200 µl with RNase-free water, and add 500 µl Simzol to mix well. Note: There is no need to add 200 µl RNase-free water and proceed directly to step 4.

2. ***Optional step:*** **If the sample contains more lipids, centrifuge at 12,000×g for 5 min. After centrifugation, a fat layer will appear on top, and the lower layer of clear solution is piped into a new 1.5 ml centrifuge tube with a tip to proceed to the next step.**
3. **Add 200 µl RNase-free water. Close the lid, shake vigorously for 15 sec.**
4. **Centrifuge at 12,000 ×g for 15 min and transfer 500 µl supernatant into a new RNase-free 1.5 ml centrifuge tube.**
5. **Add 200 µl 75% ethanol, mix well, incubate for 10 min at room temperature, and centrifuge at 12,000×g for 8 min. At this point, the large fragment RNA will form a pellet at the bottom of the tube, leaving the pellet into step 7. Transfer the supernatant containing the miRNA to a new RNase-free 1.5 ml centrifuge tube.**
6. **Add 500 µl isopropanol (about 0.8 volume), mix well, incubate at 4℃ for 30 min, centrifuge at 12,000 ×g for 15 min, and discard the supernatant. At this point, miRNA pellets will form at the bottom of the tube.**
7. **Add 1 ml 75% ethanol to the large RNA pellet (step 5) and 1 ml 70% isopropanol to the miRNA pellet (step 6), mix well, centrifuge at 8,000 ×g for 3 min, and discard the supernatant.**

   * If a large amount of RNA precipitation is observed, repeat step 7 once to reduce the residual salts in the RNA.

8. **Close the lid and spin down. Discard the remaining ethanol or isopropanol with a 200 µl tip, leaving the white RNA pellet at the bottom and walls of the tube. There is no need to dry the RNA.**

   * Note: When RNA is extracted from some samples, the RNA does not form white spots at the bottom of the tube, but rather adsorbs to the wall the tube as a uniform mist-like precipitate. Observe carefully and take special care to dissolve the RNA by adding RNase-free water to the appropriate location on the tube wall during step 9.

9. **Add 50~100 µl RNase-free water to large fragments RNA pellet (step 5) to dissolve the large fragments RNA. Add 10~50 µl RNase-free water to the miRNA pellet (step 6)to dissolve the miRNA. Store the RNA below -70℃ for later use.**

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# Protocol for total RNA extraction with RNA spin column set:

**Simzol can be used in conjunction with RNA spin column sets to simplify protocols, improve the purity of the RNA obtained, and reduce salt carryover in the RNA obtained. RNA Spin Column Set Ordering Information: Cat. No. 5302-A.**

**This protocol is designed for the extraction of total RNA by 500 µl Simzol, and the amount of Simzol added must be increased proportionally if RNA is being extracted from more tissues or cells. If RNA is extracted from trace tissues or cells (1~10 mg tissue or 102~104 cells), Carrier RNA (Simgen Cat. No. 4003101）5~10 µg is recommended to be added to Simzol. The presence of Carrier RNA does not affect RT-PCR.**

1. **Treatment for different samples sources:**

   **Animal or plant tissues/plant cells/yeasts/bacteria:**

   Grind about 100~200 mg of tissue to powder form with liquid nitrogen in a mortar, weigh 25~100 mg of tissue with a 1.5 ml centrifuge tube pre-cooled with liquid nitrogen, add 500 µl Simzol, close the lid, shake vigorously to mix well, and enter the operation of step 3.

   * Try to add Simzol before the tissue powder thaws to reduce the degradation of RNA by endogenous RNases in tissues.
   * Animal tissues can be lysed with a homogenizer or other mechanical disruption method with the addition of 500 µl Simzol.

   **Cultured animal cells:**

   **Adherent cells:** Add 500 µl Simzol per 10 $cm^2$ of cultured cells (e.g., a 3.5 cm diameter cell culture dish, discard the medium, add 500 µl Simzol directly), pipette the cells several times with the tip to lyse the cells, transfer the homogenate into a 1.5 ml centrifuge tube, and proceed to step 3.

   **Suspension cultured cells:** Centrifuge 5~10×$10^6$ cells with a 1.5 ml centrifuge tube, add 200 µl PBS solution, vortex and shake until the cells are all suspended, add 500 µl Simzol, directly pipette the cells several times with the tip to lyse the cells, note that there is no need to add 200 µl RNase-free water, directly proceed to step 4.

   **Liquid samples:**

   Add 500 µl Simzol per 200 µl liquid sample (e.g., blood, plasma, etc.), close the lid, and shake vigorously to mix. When the sample volume is less than 200 µl, make up to 200 µl with RNase-free water, and add 500 µl Simzol to mix well. Note: There is no need to add 200 µl RNase-free water and proceed directly to step 4.

2. ***Optional step:*** **If the sample contains a lot of protein, fat, polysaccharide or extracellular material (tendons, plant nodule parts, etc.), centrifuge at 12,000×g for 5 min and take the supernatant. The resulting pellet includes the cell membrane, polysaccharides, high-molecular-weight DNA, and RNA in the supernatant. When working with adipose tissue, the large amount of oil contained in the top layer should be removed. Take the cleared solution after centrifugation and proceed to the next step.**
3. **Add 200 µl RNase-free water, close the lid, shake vigorously for 15 sec, and incubate for 5 min at room temperature.**
   * For tissue sample sizes of 50 mg, it is recommended to incubate for 15 min at room temperature.
4. **Centrifuge at 12,000 ×g for 15 min and transfer 500 µl supernatant into a new RNase-free 1.5 ml centrifuge tube.**
5. **Add 280 µl absolute ethanol and mix well.**
6. **Transfer the mixture from step 5 to a spin column (the spin column is placed in a 2 ml collection tube), close the lid, and centrifuge at 12,000 rpm for 30 sec.**
7. **Discard the filtrate, place the spin column back into the 2 ml collection tube, add 800 µl Buffer WBR to the spin column, close the lid, and centrifuge at 12,000 rpm for 30 sec.**
   * The filtrate does not need to be completely discarded, if you want to avoid contamination of the centrifuge by the filtrate adhered to the mouth of the collection tube, you can slap the 2 ml collection tube upside down on a paper towel once.
   * Ensure that absolute ethanol has been added to Buffer WBR.
8. **Discard the filtrate, place the spin column back into the 2 ml collection tube, add 300 µl Buffer WBR to the spin column, close the lid, and centrifuge at 14,000 rpm for 1 min.**
   * If the centrifuge does not reach 14,000 rpm, centrifuge at full speed for 2 min.
9. **Discard the 2 ml collection tube, place the spin column to a RNase-free 1.5 ml centrifuge tube. Add 50~100 µl RNase-free Water to the spin column, close the lid, incubate at room temperature for 1 min, centrifuge at 12000 rpm for 30 sec.**

* Note: Do not let the filtrate touch the bottom of the spin column when taking out the spin column, if the spin column is contaminated with filtrate, please discard the filtrate and put the spin column back into a 2 ml collection tube and centrifuge at 14000 rpm for 1 min, then take out the spin column for this protocol.

* If the centrifuge does not have a leak-proof lid, change the centrifugation condition to 8000 rpm for 1 min to avoid the lid coming off and damaging the centrifuge.

**10. Discard the spin column, the eluted RNA can be immediately used in various molecular biology experiments or stored below -70℃ for later use.**

## Troubleshooting Guide:

### 1. RNA degradation.

**(1) Old samples are used or samples not well preserved.**

1 Extraction using fresh samples.

2 Sample Storage: Tissue samples should be snap-frozen in liquid nitrogen immediately after collection and stored below -70℃, and cell samples should be stored below -70℃ directly after collection with 500 μl Simzol. If it is not possible to store immediately below -70℃, you can purchase RNA Sample Preservation Solution (Simgen Cat. No. 4007020/4007100). When using RNA sample preservation solutions, the following should be noted:

A. Only fresh, unfrozen-thawed samples were added to the RNA sample preservation solution for preservation.

B. Do not store at room temperature for a long time after adding RNA sample preservation solution to the sample. RNA sample preservation solution has a time limit for sample preservation, generally only 1 day at 37℃, 1 week at 15-20℃, up to 4 weeks at 2-8℃, and at -20℃ or lower for long-term storage.

**(2) Contamination by exogenous RNases.**

Reagents, instruments, and RNases from the experimental environment enter the experimental system. Gloves and masks must be worn during the experiment, and RNA must be extracted in a laboratory free of RNase contamination.

**(3) Degradation occurs in the electrophoresis process.**

Formaldehyde denaturing gel electrophoresis is recommended for electrophoresis detection (see page 540 of the third edition of molecular cloning). If there are no conditions for formaldehyde denaturing gel electrophoresis, pay attention to the simgenbio WeChat public account to query the article "How to do a good RNA electrophoresis experiment" to get relevant experimental tips.

### 2. Low RNA extraction rate.

**(1) The content of polysaccharides was very high, such as the tubers, fruits, seeds and senescent leaves of some plants (mainly starchy polysaccharide derivatives), cartilage tissue (cartilage is a polysaccharide substance), and the shell crustaceans such as shrimp, crab and insect (the main component chitin is a polysaccharide). Although RNA pellet can be seen (in fact, the main pellet is polysaccharides), the yield of RNA may be very low.**

Choose Polysaccharides & Polyphenolics-rich Plant Total RNA Kit (Simgen Cat. No.5103050) to extract RNA.

**(2) The sample has low RNA content, or the sample is not sufficiently broken, or the homogenization is not complete.**

Increase the sample volume, extend the homogenization time, or mix by pipette repeatedly.

**(3) The RNA pellet is not completely dissolved.**

Increase the volume of RNase-free water and prolong the dissolution time.

### 3. DNA contamination.

**The sample contains too much DNA or uses too many sample.**

1 Reduce the amount of sample or increase the dosage of reagents such as Simzol, isopropanol, 75% ethanol, etc. proportionally.

2 Even if DNA bands are not visible on electrophoresis testing, the extracted RNA should not be assumed to be free of genomic DNA contamination. To remove DNA completely, digest residual DNA with RNase-free DNase I (to be ordered separately, Simgen, Cat. No. 8003050).

### 4. A260/A280 value is <1.6.

**(1) Too many samples and too little Simzol for homogenization lysis.**

Reduce the amount of sample or increase the amount of reagents such as Simzol, isopropanol, 75% ethanol, etc. proportionally.

**(2) Contamination of polysaccharides or proteins.**

If the sample contains a large number of polysaccharides or proteins, the supernatant can be centrifuged at 12,000 ×g for 5 min after the Simzol is added in the sample processing step, and the supernatant can be taken into step 3. The pellet obtained by centrifugation contains impurities such as extracellular membrane, polysaccharides, high molecular weight proteins, and DNA.

## 5. Follow-up experiments with RNA did not work well.

**(1) Too much RNA was used as a reverse transcription template.**

In general, 100~1000 ng of RNA is more suitable to be added to the 20 μl reverse transcription reaction system as a template. Note that cDNA needs to be appropriately diluted as a PCR template so that residual RTases, including inactivated RTases will not interfere with Taq enzyme activity.

**(2) Effect of reverse transcribed DNA-RNA complexes on fluorescent PCR.**

It is recommended to reduce the amount of random primers or reverse transcription with specific primers, or add RNase H after reverse transcription to remove the DNA-RNA complex.