Super RNApure™ Kit

Cat. #	Product	Component	Size	Preps / Kit
GB-7001	Super RNApure™ Kit	1. Super RNApure™ reagent	100 ml	100 test
		2. DF buffer	6 ml	
		3. NC buffer	6 ml	
Storage：All reagents should be kept at 4℃				

Product description

Super RNApure™ Kit is an improved reagent to isolate total RNA. This technique performs well with small quantities of tissue (50-100 mg) and cells (1-5x10^5) , and large quantities of tissue (≥1g) and cells (＞10^7), of human, animal origin, plants, yeast or bacteria (Gram positive or negative). The simplicity of the Super RNApure™ Kit method allows simultaneous processing of a large number of samples. The entire procedure will be completed in one hour. Total RNA isolated by Super RNApure™ Kit is free of DNA and protein contamination. It can be used for Northern blot analysis, in vitro translation, and RNase protection assays, RT-PCR and poly(A)$^+$selection.

The yield of total RNA depends on the sample sources. Generally, the extracted total RNA yield is about 400 – 700 ug per 100 mg of starting tissues or 50 –100 ug per 1x10^7 cells with A_{260}/A_{280} ratio about 1.7 – 2.0.

Precautions for preventing RNase contamination

- Set aside items of glassware, batches of plasticware, and buffers that are to be used only for experiments with RNA.
- Store solution/buffers in small aliquots and discard each aliquot after use. Avoid materials or stock solutions that have been used for any other purposes in the laboratory.
- Set aside special electrophoresis devices for use in the separation of RNA. Clean these devices with detergent solution, rinse in H_2O, dry with ethanol, and then fill with a 3% solution of H_2O_2, rinse the electrophoresis tank thoroughly with DEPC treated H_2O 10 minutes at room temperature.
- Prepare all solutions and buffers with RNase-free glassware, DEPC-treated water, and chemicals reserved for working with RNA that are handled with disposable spatulas or dispensed by tapping the bottle rather than using a spatula. Wherever possible, treat solutions with 0.1% DEPC for at least 1 hour at 37℃ and then autoclave for 15 minutes at 15 psi (1.05 kg/cm^2) on liquid cycle.
- Treat plasticware either with DEPC or commercially available products that inactivate RNase.
- Use disposable tips and microfuge tubes certified by a reputable manufacturer to be free of RNase. To reduce the chances of contamination, it is best to use sterile forceps when transferring these small items from their original packages to laboratory racks.
- Use inhibitors to suppress RNase during the isolation of RNA.

Instructions for RNA isolation

Caution: When working with Super RNApure™ Kit, use gloves and eye protection (shield, safety goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

Reagents Required, but not supplied

- chloroform
- isopropyl alcohol
- 75% ethanol (please prepare it with DEPC-treated water)
- DEPC-treated water

1. Homogenize sample in Super RNApure™ Reagent

a. FOR TISSUES

(1) Dissect and wash the tissue with PBS, and dry it with Kimwipes paper.

(2) To prevent RNA from degradation, put the tissue immediately in liquid nitrogen (optional).

(3) Transfer ~ 100mg of the frozen tissue or fresh tissue to a polypropylene snap-cap tube.

(4) Add 1ml of Super RNApure™ Reagent per 100 mg tissue .

(5) Homogenize the tissue at low temperature (ice bath) with a polytron or other homogenizer.

b. FOR MAMMALIAN SUSPENSION CELLS

(1) Harvest cells by centrifugation at 400xg for 5 minutes at 4℃.

(2) Remove the medium by aspiration and resuspend the cell pellet in 1 ~ 2 ml of sterile PBS.

(3) Harvest cells by centrifugation, completely remove PBS by aspiration.

(4) Add 1ml of Super RNApure™ Reagent per 10^6 ~ 10^7 cells.

c. FOR MAMMALIAN ADHESIVE CELLS

(1) Aspirate the medium and wash the dish with 10ml of PBS.

(2) Add 1.5ml of trypsin EDTA and incubate cells at 37℃ until cells detach.

(3) Spin cells at 400xg for 5 minutes at 4℃.

(4) Aspirate supernatant and resuspend cells with 1.5ml of PBS.

(5) Repeat the step (3) and aspirate PBS buffer.

(6) Add 1ml of Super RNApure™ Reagent per 10^6 ~ 10^7 cells

d. FOR LYMPHOCYTES

(1) Blood should be taken with anticoagulant, however heparin should be avoided, as it is an inhibitor of restriction enzymes. Blood may be stored for a few days at room temperature, but is best stored at 4 ℃.

(2) Add Ficoll-Paque (3 ml) to a centrifuge tube.

(3) Carefully overlay the PBS diluted blood samples (2 ml of PBS per 2 ml of blood sample) on Ficoll-Paque. (Fig. 1).

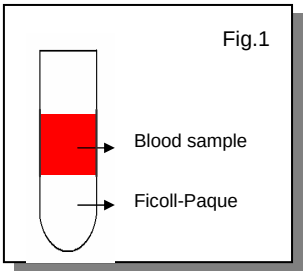
Important: **When overlaying the sample, do not mix with Ficoll-Paque.**

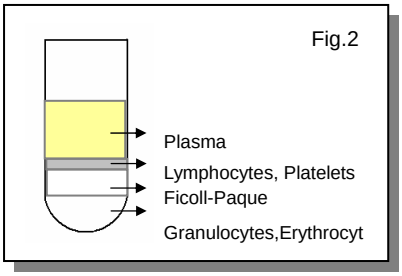
(4) Centrifuge at 400xg for 30 – 40 minutes at 18 - 20℃.

(5) Withdraw the upper layer using a clean Pasteur pipette, and leave the lymphocyte layer undisturbed at the interface (Fig.2). Care should be taken not to disturb the lymphocyte layer. The upper layer of plasma , which is essentially free of cells , may be saved for the other use.

(6) Using a clean Pasteur pipette to transfer the lymphocyte layer to a clean centrifuge tube.

(7) Add 1ml of Super RNApure™ Reagent per 10^6 ~ 10^7 cells





2. Separate Phases

(1) Add 50ul of DF buffer, and mix well by inverting the tube.

(2) Add 100ul of chloroform and vortex thoroughly.

(3) Incubate the tube on ice for 15 minutes.

(4) Centrifuge the tube at 12,000xg for 15 minutes at 4℃, then transfer the upper aqueous phase containing the extracted RNA to a fresh tube.

3. Precipitate RNA

(1) Add 50 ul of NC buffer and 600ul of isopropanol (isopropyl alcohol) to the RNA solution and vortex.

(2) Spin the RNA at 12,000xg for 10 minutes at 4℃.

4.Wash and Solubilize

(1) Carefully withdraw the supernatant, wash the pellet twice with 600ul 75% alcohol, centrifuge again to spin down RNA pellet, and remove any remaining ethanol.

(2) Air dry the RNA pellet. Do not allow the pellet to dry completely.

(3) Resuspend RNA with 50 – 100 ul of DEPC treated water.

(4) Store the RNA solution in deep freezer (-80℃).

Optional：

- For fat tissues, muscles, or some solid tissues, an additional isolation step may be required, because high content of proteins, fat, polysaccharides, or extracellular material are present. Following homogenization, remove insoluble material from the homogenate by centrifugation at 12,000xg for 10 minutes at 2 ~ 8℃. The resulting pellet contains extracellular material, membranes, polysaccharides, and high molecular weight DNA, while the supernatant contains RNA.
- For yeast, bacteria (Gram positive or negative), the pretreatment is needed. Centrifuge the 3 ml of bacteria or yeast (OD_{600} of 0.6-1.0) culture. Resuspend the pellet in 200 ul of lysozyme (5 mg/ml 1X TE buffer). Add 20 ul of 10% Triton X-100. Incubate for 20 minutes at 37℃. Add 1 ml Super RNApure reagent to the 220ul resuspension. The other steps are the same as the standard procedure.
- For plants, the pretreatment is needed. Freeze leaf tissue in liquid nitrogen and grind using a mortar and pestle. Lyse 50 mg tissue powder in 1 ml of Super RNApure™ Reagent. The other steps are the same as the standard procedure.

Troubleshooting Guide:

- **The expected RNA yields per 100mg tissues or culture cells**

  Heart, 100-200ug.

  Kidney: 300-400ug

  Liver and spleen, 600-1000ug

  Fibroblasts, 10-50ug/1x10^7
- **If low yield happens, the cause may be:**

  RNA incomplete resuspension.

  Incomplete tissue homogenization.
- **If A_{260}/A_{280}<1.65, the cause may be:**

  RNA is measured in water instead of TE.

  The Super RNApure™ Reagent is not enough in the first treatment.

  Phenol contamination (be careful about the interphase).

  RNA incomplete resuspension.
- **If RNA degradation happens, the cause may be:**

  Samples were not preserved in –80℃ if not processed immediately.

  Samples were treated too long by trypsin digestion.

  Apparatuses were not DEPC-treated.

  Formaldehyde used in agarose-gel has low pH.
- **If DNA contamination happens, the cause may be:**

  The Super RNApure™ Reagent is not enough in the first treatment.

  Samples contain solvent, strong buffer and high pH.
- **If proteoglycan and polysaccharide contamination, problems can be resolved by:**

  In some tissues, polysaccharide or proteoglycan interferes subsequent resuspension and RNA analysis. Add to the aqueous phase 0.25ml of isopropanol followed by 0.25ml of a high salt precipitation solution (0.8M sodium citrate and 1.2M NaCl) per 1ml of Super RNApure™ Reagent used for the homogenization. Mix well and spin the solution to isolate RNA as described in the protocol. Thus this change will leave the proteoglycan and polysaccharide contamination in a solution and RNA in pellet without compromising the recovery rate of RNA purification.

Super RNApure™ kit protocol

Homogenize cells or tissue in 1ml of **Super RNApure™ solution** and vortex

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Add 50ul of **DF** buffer solution and vortex

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Add 100ul of Chloroform and vortex

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Keep on ice. (15 minutes)

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Centrifuge (12,000xg, 15 minutes,4℃)

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Take aqueous phase；
Add 50ul of **NC** buffer solution and 600ul of Isopropanol and vortex

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Centrifuge (12,000xg, 10 minutes,4℃)

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Pellet RNA and wash with DEPC-treated ethanol

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Centrifuge (7,500xg, 5 minutes,4℃)

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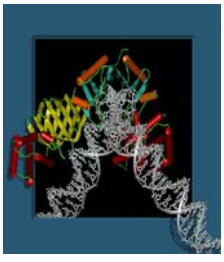
Dry pellet and dissolve in DEPC-treated water

Elapsed Time＜ 1 h



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