Geneaid

Instruction Manual
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For Research Use Only

rSYNC™ RNA Isolation Kit

RS004, RSD004 (4 Preparation Sample Kit)
RS050, RSD050 (50 Preparation Kit)
RS100, RSD100 (100 Preparation Kit)
RS300, RSD300 (300 Preparation Kit)

Advantages

Sample: tissue, fresh whole human blood, cultured cells
Yield: up to 50 µg of RNA
Format: RNA spin column
Operation Time: within 20 minutes
Elution Volume: 25-100 µl
Kit Storage: dry at room temperature (15-25°C)

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Introduction

The rSYNC™ RNA Isolation Kit was designed specifically for purifying total RNA from a variety of samples, such as fresh whole human blood, cultured animal cells, animal tissues. Detergents and chaotropic salt are used to lyse cells and inactivate RNase with a optional DNase treatments. RNA in the chaotropic salt is bound by the glass fiber matrix of the spin column and once contaminants have been removed, the purified total RNA is eluted by RNase-free Water. High quality total RNA can be purified in less than 20 minutes without phenol extraction or alcohol precipitation. The purified RNA is ready for use in RT-PCR, northern blotting, primer extension, mRNA selection and cDNA synthesis.

Quality Control

The quality of the rSYNC™ RNA Isolation Kit is tested on a lot-to-lot basis according to Geneaid's ISO-certified quality management system. Total RNA is isolated from a 25 mg animal tissue sample, quantified with a spectrophotometer and analyzed by electrophoresis.

Kit Components

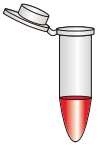
Component	RS004 RSD004	RS050 RSD050	RS100 RSD100	RS300 RSD300
RBC Lysis Buffer	10 ml	100 ml	200 ml	500 ml
RS Buffer	2 ml	30 ml	60 ml	130 ml
DNase I (2U/ µl)[1] (RSD004/050/100/300)	20 µl	275 µl	550 µl	550 µl x3
DNase I Reaction Buffer (RSD004/050/100/300)	200 µl	2.5 ml	5 ml	15 ml
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer[2] (Add Ethanol)	1.5 ml (6 ml)	25 ml (100 ml)	25 ml+ 12.5 ml (100 ml+ 50 ml)	50 ml x2 (200 ml x2)
RNase-free Water	1 ml	6 ml	15 ml	30 ml
RB Column	4 pcs	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	8 pcs	100 pcs	200 pcs	600 pcs

[1]DNase I is shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.

[2]Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. Additional Wash Buffer x 12.5 ml is included with RSD100 only.

During the procedure, always wear a lab coat, disposable gloves, and protective goggles.

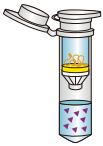
Quick Protocol Diagram



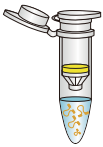
RBC lysis of whole blood samples

Cell lysis of leukocyte, cultured cell and tissue samples

RNA binding to membrane while contaminants remain suspended



Wash (removal of contaminants while RNA remains bound to membrane)



Elution of pure total RNA which is ready for subsequent reactions

rSYNC™ RNA Isolation Kit Protocol

Please read the entire instruction manual prior to starting the Protocol Procedure.

IMPORTANT BEFORE USE!

1. DNase I is shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.

2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

3. Prepare Phosphate Buffered Saline (PBS, pH7.2) and 0.10-0.25% Trypsin for cultured cells.

4. Yield and quality of RNA will be higher when fresh samples or samples which have been flash frozen and stored at -70ºC are used.

Additional Requirements

Absolute ethanol and ddH_2O (RNase/DNase-free) to prepare 70% ethanol, ß-mercaptoethanol or twice the required volume of freshly prepared 2M Dithiothreitol in RNase free Water, microcentrifuge tubes. For animal tissue samples: tissue homogenizer or mortar, pestle and 20-G needle syringe.

Sample Preparation

1. Blood

Collect fresh human blood in anticoagulant-treated collection tubes. Add **1 ml of RBC Lysis Buffer and 300 µl of whole human blood** to a sterile 1.5 ml microcentrifuge tube. Mix by inversion. Incubate the tube on ice for 10 minutes (briefly vortex twice during incubation). Centrifuge at 3,000 x g for 5 minutes then remove the supernatant completely. Add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol**. Resuspend the cells by pipetting then incubate at room temperature for 5 minutes.

2. Adherent Cultured Animal Cells

A. Cell lysis in a culture dish

Aspirate the culture medium completely. Add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol** immediately to the culture dish (up to 5 $x10^6$ cells). Incubate at room temperature for 5 minutes then transfer the cell lysate to a 1.5 ml microcentrifuge tube.

B. Trypsinize cell prior to cell lysis

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal Cells.

3. Suspension Cultured Animal Cells

Transfer **cells (up to 5 x 10^6)** to a 1.5 ml microcentrifuge tube or 15 ml centrifuge tube. Harvest by centrifugation for 5 minutes at 300 x g then remove the supernatant. Add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol**. Resuspend cells by pipette. Incubate at room temperature for 5 minutes.

4. Tissue

Cut off **10-25 mg of fresh/frozen tissue**. Do not use more than 25 mg of tissue per reaction. If using frozen animal tissue, samples must have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use to avoid RNA degradation.

Homogenize tissue using one of the following methods

A. Transfer tissue to a suitably sized vessel. Add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol**. Disrupt and homogenize the tissue using a conventional rotor–stator homogenizer until it is uniformly homogeneous. Transfer the lysate to a 1.5 ml microcentrifuge tube. Incubate the sample lysate at room temperature for 5 minutes then centrifuge at 12-16,000 x g for 2 minutes. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

B. Transfer tissue to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads. Add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol**. Homogenize the sample with a TissueLyser, Disruptor Genie or similar. Incubate at room temperature for 5 minutes then centrifuge at 12-16,000 x g for 2 minutes. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

C. Freeze tissue in liquid nitrogen then grind thoroughly with a mortar and pestle. Transfer the tissue powder to a 1.5 ml microcentrifuge tube (do not allow the tissue to thaw) then add **400 µl of RS Buffer and 4 µl of ß-mercaptoethanol**. Shear the tissue by passing the lysate through a 20-G needle syringe 10 times. Incubate the sample lysate at room temperature for 5 minutes then centrifuge at 12-16,000 x g for 2 minutes. Transfer the supernatant to a clean 1.5 ml microcentrifuge tube.

RNA Binding

Add **1 volume of 70% ethanol prepared in ddH_2O (RNase and DNase-free)** and shake the mixture vigorously. Place a **RB Column** in a 2 ml Collection Tube and transfer **500 µl of the mixture** to the **RB Column**. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Transfer the remaining mixture to the same RB Column then centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and place the RB Column in a new 2 ml Collection Tube.

Optional Step 1: In Column DNase I Digestion

DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.
2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.
3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNase I solution to mix (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.
5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with the RNA Wash step.

RNA Wash

Add **400 µl of W1 Buffer to the RB Column** then centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. **Add 600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer (make sure ethanol was added)** into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

RNA Elution

Place the **dried RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). **Add 50 µl of RNase-free Water** into the **CENTER** of the column matrix. Let stand for at least 3 minutes to ensure the **RNase-free Water** is absorbed by the matrix. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0). Incubate the microcentrifuge tube at 65°C for 10 minutes.

NOTE: DNase I Reaction Buffer may cause aberrant migration or smearing of RNA on gels. If analyzing RNA by gel electrophoresis, repurify the RNA sample by using the Geneaid™ RNA Cleanup Kit instead of stopping the reaction with EGTA.

Troubleshooting

Clogged Column: Reduce the amount of starting material or separate it into multiple tubes. Centrifugation temperature must be between 20ºC to 25ºC. For animal tissue samples, after homogenization, centrifuge the sample lysate at 2-16,000 x g for 2 minutes to remove the cell debris.

DNA contamination: Perform In Column DNase I Digestion or DNA Digestion In Solution step to eliminate DNA contamination.

Residual Ethanol Contamination: Following the wash step, dry the RB Column with additional centrifugation at 14-16,000 x g for 5 minutes.

Degraded RNA: Use fresh samples or freeze samples at -70 ºC immediately after collection. Extracted RNA should be stored at -70ºC.

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