rSYNC™ RNA Isolation Kit Quick Protocol

For research use only

Catalogue Number

RS004, RSD004, RS050, RSD050, RS100, RSD100, RS300, RS300

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

IMPORTANT BEFORE USE!

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

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 (See complete protocol)

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 (see complete protocol)

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 (12.5 ml) is included with RSD100 only.

Storage: dry at room temperature (15-25ºC)