FastPure Cell/Tissue Total RNA Isolation Kit V2

NB-54-0206

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Cat# NB-54-0206

Product Description

The FastPure® Cell/Tissue Total RNA Isolation Kit V2 provides a rapid method for extracting total RNA from animal tissues and cells. It works based on silica membrane purification technology and does not require β-mercaptoethanol, phenol/chloroform, or any other toxic reagent during the extraction process. All it takes is 6 minutes to extract high-quality RNA. The kit contains FastPure gDNA-Filter Columns III which can effectively remove impurities and gDNA. FastPure RNA Columns III can efficiently bind RNA and are used together with an optimized buffer to produce high-purity total RNA. The isolated RNA contains little residual gDNA and is free from proteins or other contaminating impurities. It can then be used for various downstream experiments, including RT-PCR, real-time PCR, and microarray analysis.

Components

Component	NB-54-0206 (50 rxns)
Buffer RL	35 ml
Buffer RW1	45 ml
Buffer RW2	20 ml
RNase-free ddH_2O	10 ml
FastPure gDNA-Filter Columns III (each in a 2 ml Collection	50 units
Tube)FastPure RNA Columns III (each in a 2 ml Collection Tube)	50 units
RNase-free Collection Tubes 1.5 ml	50 units

Buffer RL: Provides the environment needed for animal tissue and cell lysis;
Buffer RW1: Removes impurities such as proteins and DNA;
Buffer RW2: Removes salt ion residues;
RNase-free ddH2O: Elutes total RNA;
FastPure gDNA-Filter Columns III: Adsorb DNA and remove impurities in the lysate;
FastPure RNA Columns III: Specifically adsorb RNA;
Collection Tubes 2 mL: Collect filtrate;
RNase-free Collection Tubes 1.5 mL: Collect RNA.

Storage

Store at 15–25°C and transport at room temperature.

Application

10–20 mg of animal tissue
<5 × 106 cultured cells

Self-prepared Materials

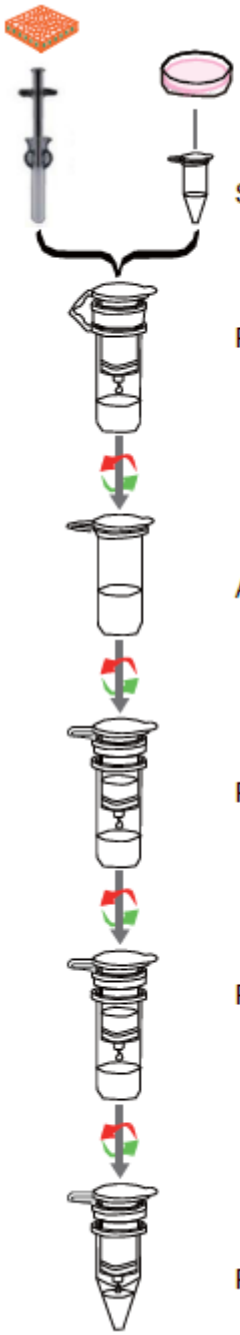
Anhydrous ethanol, 50% ethanol, 1.5 mL RNase-free centrifuge tubes, RNase-free pipette tips, etc.

Notes

1. Before first use, please refer to the Buffer RW2 bottle label and add 80 mL of anhydrous ethanol to Buffer RW2. Label accordingly.
2. Check for any crystallization in Buffer RL and Buffer RW1 before use. If crystals have formed, allow them to dissolve at 37°C and mix well before use.
3. Keep the amount of sample to be processed to 10–20 mg of tissue or <5 × 106 cells. Do not use more than 10 mg of tissue rich in DNA/RNA, such as that of the liver, spleen, and kidneys. Using more than this amount may result in residual gDNA or reduced yield in addition to blockage of the FastPure gDNA-Filter Column III.

4. Use fresh samples. If extraction is not performed immediately, quickly freeze the sample in liquid nitrogen and store at -85 to -65°C. Avoid repeated freezing and thawing. Alternatively, homogenize the sample immediately in Buffer RL and store at -85 to -65°C. Sample collection and storage should be carried out as quickly as possible to prevent RNA degradation.
5. Incomplete disruption of the sample affects RNA yield and is likely to cause blockage in the column. Samples should be homogenized at a controlled temperature as much as possible to prevent RNA degradation due to high temperatures.
6. For extraction of RNA from liver tissue, please read **Experiment Process** carefully.
7. When using this kit, please wear a laboratory coat, disposable latex gloves, and a disposable mask. Use RNase-free consumables to prevent RNase contamination.
8. All procedures must be carried out at room temperature (15–25°C).

Mechanism & Workflow



Sample lysis: 500 µL Buffer RL

Removal of gDNA: Transfer the lysate to a FastPure gDNA-Filter Column III and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. **Collect the filtrate.**

Adjustment of upper column: Add 0.5 times the filtrate volume of anhydrous ethanol to the filtrate and mix well.

RNA adsorption: Transfer the entire mixture into a FastPure RNA Column III and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. **Discard the filtrate.**

Removal of impurities: Add 700 µL of Buffer RW1 and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. Discard the filtrate.
Add 700 µL of Buffer RW2 (with anhydrous ethanol added) and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. Discard the filtrate.
Add 500 µL of Buffer RW2 (with anhydrous ethanol added) and centrifuge at 12,000 rpm (13,400 × g) for 2 minutes. Discard the collection tube.

RNA elution: Add 50–200 µL of RNase-free ddH_2O and centrifuge at 12,000 rpm (13,400 × g) for 1 minute.

Experiment Process

Please read this procedure carefully before starting the experiment.

- Before first use, please refer to the bottle label of Buffer RW2 and add 80 mL of anhydrous ethanol to Buffer RW2. Label accordingly.
- For liver tissue samples, 50% ethanol is required. Prepare it in advance using RNase-free ddH2O.
- Check for any crystallization in Buffer RL and Buffer RW1 before use. If crystals have formed, allow them to dissolve at 37°C and mix well before use.

Sample Processing

Animal Tissue

- Homogenization: Add 500 µL of Buffer RL to every 10–20 mg of fresh tissue (350 µL for liver tissue). Homogenize using a glass or electric homogenizer until there are no visible tissue pieces.

Perform the homogenization on ice so that the RNA does not degrade due to transient, localized increases in temperature.

- Grinding using liquid nitrogen: After grinding the sample using liquid nitrogen, immediately transfer to Buffer RL, adding 500 µL of Buffer RL for every 10–20 mg of pulverized sample (350 µL for liver tissue). Vortex until there are no visible clumps of powder.

If extraction is not performed immediately, keep homogenized samples or samples pulverized with liquid nitrogen at -85 to -65°C.

Cultured cells

- Adherent cells: No digestion is required. Remove the supernatant from the culture medium and add Buffer RL directly to the culture dish for digestion and lysis. Alternatively, collect trypsinized cells by centrifugation and add 500 µL of Buffer RL to every <5 × 106 cells. Vortex until there are no visible clumps of cells.
- Suspending cells: Collect the cells directly by centrifugation. Add 500 µL of Buffer RL to every <5 × 106 cells and vortex until there are no visible clumps of cells.

If extraction is not performed immediately, keep the cell lysate at -85 to -65°C.

RNA Extraction

Perform the following steps in an RNase-free environment.

1. Place the FastPure gDNA-Filter Column III into a Collection Tube and transfer the lysed sample into the column. Centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. Discard the FastPure gDNA-Filter Column III and collect the filtrate.
2. Add 0.5 times the filtrate volume of anhydrous ethanol to the filtrate (the same volume of 50% ethanol for liver tissue samples) and mix well.

It is normal for the solution to turn turbid or to contain flocculent precipitate after adding ethanol. Mix the solution well by vortexing and use it directly for the next step.

3. Transfer the entire mixture from Step 2 to a FastPure RNA Column III (placed in a Collection Tube). Centrifuge at 12,000 rpm (13,400 × g) for 30 seconds and discard the filtrate.
4. Add 700 µL of Buffer RW1 to the FastPure RNA Column III and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. Discard the filtrate.
5. Add 700 µL of Buffer RW2 (with anhydrous ethanol added) to the FastPure RNA Column III and centrifuge at 12,000 rpm (13,400 × g) for 30 seconds. Discard the filtrate.
6. Add 500 µL of Buffer RW2 (with anhydrous ethanol added) to the FastPure RNA Column III and centrifuge at 12,000 rpm (13,400 × g) for 2 minutes. Carefully remove the adsorption column from the collection tube without touching the filtrate so as to prevent contamination.
7. (Optional) If the adsorption column contains residual liquid or has touched the filtrate, discard the filtrate and place the FastPure RNA Column III back into the collection tube. Centrifuge at 12,000 rpm (13,400 × g) for 1 minute to prevent ethanol contamination.
8. Carefully transfer the adsorption column to a new 1.5 mL RNase-free Collection Tube. Add 50–200 µL of RNase-free ddH2O to the center of the adsorption column without touching the membrane and allow the tube to stand at room temperature for 1 minute. Centrifuge at 12,000 rpm (13,400 × g) for 1 minute to elute the RNA.

Pre-warming the RNase-free ddH2O to 65°C can help increase the yield. After adding the RNase-free ddH2O dropwise to the membrane, allow the tube stand at room temperature for 2–5 minutes or elute the RNA again after centrifugation.

9. The extracted total RNA can be directly used for downstream experiments or stored at -85 to -65°C.

FAQ & Troubleshooting

Common Issues	Reason	Solution
Blockage of the adsorption column	1. Too much sample was added	Reduce the amount of sample added. Do not add more than 10 mg of tissue from the liver, spleen, or kidney, which are rich in DNA/RNA.
	2. The sample is rich in muscle fibers	Process the sample by grinding it with liquid nitrogen. Increase the intensity of grinding for tissues rich in muscle fiber, such as muscular, cardiac, and skin tissue.
	3. The sample was inadequately ground or homogenized	Centrifuge the lysed sample at 12,000 rpm (13,400 × g) for 5 minutes, and then collect the supernatant for subsequent extraction.
No RNA is extracted or the yield is low	1. Too little sample was added	Increase the amount of sample added. Do not exceed 20 mg of tissue or 5×10^6 cells.
	2. The sample was not stored properly	Intrinsic RNase has degraded the RNA. Use fresh samples or samples at -85 to -65°C that have never been thawed.
	3. The sample was inadequately ground or homogenized	Increase the volume of lysis buffer and lysis time.
	4. Elution was not done properly	Add RNase-free ddH_2O to the center of the membrane and reduce the elution volume as appropriate. Alternatively, pre-warm the RNase-free ddH_2O to 65°C, leave the tube at room temperature for a longer time, or elute a second time.
RNA degradation	1. The sample was not promptly stored	Use fresh samples or samples at -85 to -65°C that have never been thawed.
	2. Repeated freezing and thawing of sample	Avoid freezing and thawing the sample repeatedly and store in aliquots.
	3. Contamination from electrophoresis or from the environment	Before the electrophoresis, soak the electrophoresis tank in 3% hydrogen peroxide for 20 minutes and rinse it with RNase-free ddH_2O. Ensure that the electrophoresis buffer was prepared using RNase-free ddH_2O and that the extraction was performed in an RNase-free environment.
Inhibition of drift or low purity	1. Salt ion residues	Ensure that elution with Buffer RW2 is performed twice. Additionally, add Buffer RW2 to the sides of the adsorption column or close the lid of the column and invert 2 to 3 times after adding Buffer RW2. This can help to completely wash away any salts on the sides of the column.
	2. Ethanol residue	Perform optional Step 7.
Contamination with gDNA	1. Too much sample was added	Different types of samples contain very different amounts of DNA/RNA. Do not use more than 20 mg oftissue or 5×10^6 cells. Tissue samples that are rich in DNA, such as those from the liver, spleen, and kidney,should not be used in amounts exceeding 10 mg. Residual gDNA can be further removed by digestionusing DNase. For details, please contact NeoBiotech's technical support (tech@neo-biotech.com).