RESEARCH INSTRUMENTS

Enabling Next-Generation Science

PRODUCT INFORMATION

Pure·NA

PureNA™ Fastspin Total RNA Extraction Kit
Cat# KN04-50, KN04-250

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Kit Components

Cat#	KN04-50	KN04-250
Components	**50 preps**	**250 preps**
Solution R1	10 mL	50 mL
Solution R2	30 mL	75 mL x 2
Wash Buffer	15 mL (add 45 mL ethanol before use)	37.5 mL x 2 (add 112.5 mL ethanol before use)
Elution Buffer	10 mL	50 mL
Spin Columns	50	250

Storage and Transportation

1. *PureNA*™ Fastspin Total RNA extraction kit can be stored at room temperature (15~25°C) for up to 24 months.
2. The kit can be transported at room temperature.

Description

PureNA™ Fast Total RNA extraction kit is a ready-to-use reagent for rapid isolation of total RNA from whole blood, animal and plant tissue, cultured cells and bacteria. The kit provides a very simple, fast and economical technique to isolate high quality RNA. The pure RNA can be applied extensively in Northern blot, blotting hybridization, poly(A)+ selection, *in vitro* translation, RNase protect assay, RT-PCR / Real time RT-PCR analysis, construction cDNA library and other RNA-based analyses.

Technical Information

Method	Processing Time	Max volume to be added into spin column	Yield
Spin column	7~15 mins	750 µL	≥90%

(ii) **White blood cell**:
Centrifuge the fresh blood cells at 3,000 rpm for 10 mins and collect the layer of white blood cells. Alternatively you can separate the leukocytes using "Red Blood Cell Lysis Buffer" or "Lymphocytes Separation Medium" from whole blood.

Transfer 100 µL / 2 x 10^6 cells to a new 1.5mL tube. Add 100 µL solution R1 and mix thoroughly for 30 sec. Incubate the mixture at room temperature for 1 min before proceeding to Step 2.

(iii) **Tissues**:
Using a sample of tissues of <30 mg, grind the fresh or frozen tissues in liquid nitrogen, and transfer sample to a new 1.5 mL microcentrifuge tube. Proceed to Step 2.

(iv) **Mammalian Cultured cells**:

(a) Suspension cells: Collect the cell pellets (100 µL / 2 x 10^6 cells) after centrifugation of the cells for 2 mins at 3000 rpm. Discard the supernatant. Add 100 µL solution R1 and mix for 30 sec. Incubate at room temperature for 1 min before proceeding to Step 2.

(b) Adherent cells: Transfer 100 µL / 2 x 10^6 cells into a new microcentrifuge tube (not provided). Add 100 µL of solution R1 and mix thoroughly for 30 sec. Incubate the mixture at room temperature for 1 min before proceeding to Step 2.

(v) **Body fluids and another liquid sample** (urine, ascites, hydrothorax, hydrocephalus and other body fluids sample.): Use 100 µL of sample directly in Step 2.

(vi) **Bacteria**:
Transfer bacteria cultures (at most 2 x 10^6 cells) to a 1.5 mL microcentrifuge tube. Collect the cell pellet by centrifugation for 1 min at 5,000 rpm. Discard the supernatant, leaving the pellet as dry as possible. Add 100 µL solution R1 and mix thoroughly for 30 sec. Incubate the mixture at room temperature for 1 min before proceeding to Step 2.

2. Add 600 µL of solution R2 and mix thoroughly. Incubate at room temperature for 3-5 mins.
3. Transfer the supernatant into a spin column and centrifuge the mixture for 30 sec at 10,000 rpm. Discard the flow-through.
4. Add 600 µL wash buffer into the spin column and centrifuge at 10,000 rpm for 30 sec.
5. Repeat step 4. Centrifuge for an additional 1 min at 10,000 rpm.

6. Transfer the spin column to a fresh, sterile 1.5 mL microcentrifuge tube. Add 30-50 µL Elution Buffer (or RNase-free water pH >7.0) to the centre of the membrane. Incubate at room temperature for 1 min and centrifuge for 30 sec to elute RNA. Discard the spin column.
7. Use the purified RNA immediately for downstream applications or store RNA at -20°C or -80°C for long term storage.

Analysis of DNA

1. Absorbance analysis of yield and purity

a) Prepare RNA sample by diluting it using 25 mM Tris-HCl (pH 7.5), RNase-free water or TE buffer in the correct proportion.

b) Zero the spectrophotometer at 260 nm and 280 nm with 25 mM Tris-HCl, RNase-free water or TE buffer;

c) Measure the OD_{260} using 100 µL of the RNA diluent solution, calculating the concentration of RNA as follows:
Final concentration = (Spec reading A_{260}) × (Dilution factor) × (Conversion factor A_{260})

The conversion factor for RNA is 0.040 µg/µL per OD_{260} unit
Note: To ensure significance, readings should be ≥ 0.10 and ≤ 1.0

d) Calculate the purity of RNA as follows: Purity Ratio = (Spec. reading A_{260}) / (Spec. reading A_{280})

Purity Ratio of RNA should ideally fall within the range of 1.8~2.0.
(Low pH will alter the OD measurements between 260 nm and 280 nm, erroneously indicating a low purity as RNA remains stable at pH 7.5-8.5.)

Troubleshooting

1. Low RNA yield
Ensure that 95% ethanol was added to wash buffer, and mixed evenly.

2. Degraded RNA
In order to avoid RNase contamination, wear gloves and clean all tips and micro centrifuge tubes with DEPC - ddH_2O. If contamination persists, purify the samples in laminar flow cabinet.

3. Column Clogging
- Sometimes white floccules appear after adding Solution R2 buffer. This is the result of DNA precipitation, which should not influence the isolation procedure.
- To prevent this from happening, you may decrease cell density to 2 x 10^7 cells / mL or lower. Vortex the suspension thoroughly before add Solution R2 buffer. Add Solution R2 for a while and shake.

4. Extra Residue

If there is residual liquid left on the tube after transferring the supernatant to the spin column and centrifuging for 30 sec centrifugation, it may mean that an excessive amount of sample has been used, or that the lysis step is incomplete.

This problem can be resolved using the following methods:

a) Reduce the amount of starting material.
b) Mix thoroughly after adding Solution R2 buffer.

Volume of samples before treatment

Sample	Amount
Blood	≤100 µL
Animal/plant tissue	3~30 mg
Cultured cell	≤2×10^6 cell
Body fluid	1~10 mL
Bacteria	1 mL

Apparatus and materials to be supplied by the user

- Sterile 1.5 mL microcentrifuge tubes
- Absolute ethanol (≥95%)
- Microcentrifuge capable of 14,000 rpm
- Vortex mixer

IMPORTANT NOTES

1. Add ethanol (according to volume specified on bottle label) to the wash buffer and mix them well.
2. The kit should remain stable for 24 months if stored at room temperature.
3. Centrifuge the samples at 12,000 rpm — 14,000 rpm at room temperature unless specific speed is mentioned. (Perform the centrifugation at 4°C if possible).
4. Ensure that 30 µL-100 µL of elution buffer is added to the center of the membrane. This will ensure that the membrane is soaked fully.
5. The extracted RNA should be stored at -20°C or -80°C for downstream application. Long term storage is not advised, as RNA degrades extremely easily. RNA sample should also be stored in liquid nitrogen or stock buffer in order to ensure purity of RNA.

Procedure

1. Disruption and homogenization for different starting materials:

   (i) **Whole blood**:

   Add whole blood sample (≤100 µL) into a 1.5 mL microfuge tube. Add same volume of solution R1 and mix well. Proceed to Step 2.

   If the volume of the blood sample is more than 100 mL, please treat the sample with "Red Blood Cell Lysis Buffer" or "Lymphocytes Separation Medium" to obtain leukocytes and extract suspended cells according to the following method.