11. Centrifuge at 12,000 rpm for 60 sec. Remove the Spin column and discard it.
12. Use the purified RNA for downstream applications. Store the RNA at -80°C if it is not used immediately.

Troubleshooting

Low RNA yield

RNA may be affected by oxidation during extraction and the oxidation leads to low yield or yield instability. This is most apparent in oxidase rich samples. Adding β-Mercaptoethanol into the RLysis Buffer (at a ratio of 20 µL β-ME : 1 mL RLysis Buffer) will suppress the impairment effectively. After adding β-ME, the RLysis Buffer should be stored at 4°C.

Insufficient Digestion

DNase I is reacting in the membrane, avoiding RNase contaminant exactly. The mixture of DNase Buffer, $MnCl_2$ and DNase I should not be incubated in low temperature after being mixed to avoid the insufficient digestion which because of low temperature.

Degraded RNA

In order to avoid RNase contamination, wear gloves and clean all tips and micro centrifuge tubes with DEPC-ddH_2O. If condition persists, please operate the purification in ultra-clean cabinet.

RESEARCH INSTRUMENTS
Enabling Next-Generation Science

Pure·NA

PRODUCT INFORMATION

PureNA™ Biospin Total RNA Extraction Kit
Cat# KN05-50, KN05-250

Kit Components

Cat#	KN05-50	KN05-250
Components	50 preps	250 preps
RLysis Buffer	8.75 mL	43.75 mL
RD Buffer	17.5 mL	87.5 mL
DNase Buffer	2 mL	10 mL
$MnCl_2$	450 µL	2.25 mL
DNase I	50 µL (stored at -20°C)	250 µL (stored at -20°C)
DNase Stop Buffer	5 mL (add 6.5 mL ethanol before use)	25 mL (add 32.5 mL ethanol before use)
Wash Buffer	32 mL (add 48 mL ethanol before use)	80 mL x 2 (add 120 mL ethanol before use)
RElution Buffer	10 mL	50 mL
Spin columns	50	250

Storage and Transportation

- *PureNA*™ Biospin Total RNA Extraction kit can be stored at room temperature (15-25°C) for up to 18 months. However, DNase I should be stored at -20°C.
- The kit can be transported at room temperature.

Description

PureNA™ Biospin Total RNA Extraction kit is a ready-to-use reagent for the isolation of total RNA from animal tissues, cells, bacteria and other samples (plant tissue is not recommended).

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, *in vitro* translation, RNase protect assay, RT-PCR and Real time RT-PCR analysis, cDNA library construction as well as other RNA-based analyses.

Apparatus and materials to be prepared by the user

- Sterile 1.5 mL centrifuge tubes
- Alcohol (≥95%)
- β- mercaptoethanol
- Vortex mixer
- Microcentrifuge capable of 14,000 rpm

IMPORTANT NOTES

1. Add alcohol according to the volume specified on bottle label to DNase Stop Buffer and mix well.
2. Add alcohol according to the volume specified on bottle label to Wash Buffer and mix well.
3. Centrifuge the samples at 12,000 rpm—14,000 rpm at room temp (if possible perform all the steps at 4°C).

Procedure

1. Add 175 µL RLysis Buffer and ≤30mg liquid nitrogen grinded sample into a 1.5 mL or 2.0 mL centrifuge tube and mix well.
   For liquid sample, mix 75 µL RLysis Buffer and 100 µL sample in a tube.
2. Add 350 µL RD Buffer and mix well. Incubate the mixture at 70°C for 4 mins.
3. Centrifuge the mixture at 12,000 rpm for 10 mins, and transfer the supernatant into a new tube.
4. Add 200 µL absolute alcohol and mix well. Transfer the mixture into a Spin column and centrifuge the spin column at 12,000 rpm for 60 sec.
5. Add 600 µL Wash Buffer into the Spin column. Centrifuge at 12,000 rpm for 30 sec and discard the flow-through.
6. Add the mixture of 40 µL DNase Buffer, 9 µL $MnCl_2$ and 1 µL DNase I into the Spin Column. Incubate in room temperature for 15 mins.
7. Add 200 µL DNase Stop Buffer into the Spin column. Centrifuge at 12,000 rpm for 30 sec and discard the flow-through.
8. Add 600 µL Wash Buffer into the Spin column. Centrifuge at 12,000 rpm for 30 sec and discard the flow-through.
9. Add 250 µL Wash Buffer into the Spin column. Centrifuge at 12,000 rpm for 60~120 sec and discard the flow-through. Transfer the Spin column to a new and sterile 1.5 mL centrifuge tube (RNase-free).
10. Add 50~100 µL RElution Buffer (or RNase-free water pH >7.0) to the centre of the Membrane. Incubate the tube at the room temperature for 1 min.