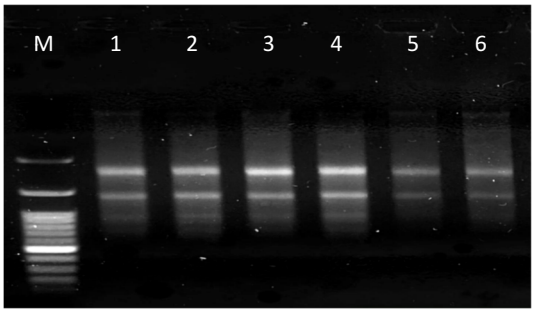
Total RNA Extraction Kit – *Tissue*

Cat. No. RT10

For total RNA extraction from Tissue

Biomate™ ***Total RNA Extraction Kit – Tissue*** *is designed by patented technology for purifying total RNA from varied animal tissues. This method uses detergents and a chaotropic salt to lyse cells and inactivate RNase. RNA in the chaotropic salt solution binds to the glass fiber matrix (NAB Filter) of PALL NAB Nanosep® Device. Following washing off contaminants, purified RNA is eluted by RNase-free water. ssRNA and dsRNA can be efficiently purified. Purified RNA is ready for RT-PCR, northern blotting, primer extension and cDNA library construction.*



Total RNA extracted from Mouse tissue

Total RNA from 10 mg of varied mouse tissue samples was extracted by *Biomate™* ***Total RNA Extraction Kit – Tissue***. 10 µl of 60 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1.5 % agarose gel.

1: Kidney	2: Spleen	3: Thymus
4: Liver	5: Muscle	6: Lung

Precautions

- **For research use only.**
- **Handling Requirements:**
  When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
- **Waste Handling:**
  Handle waste according to the country, federal, state and local regulations.
- **Do not use the product if it has expired.**

Kit Components

- RL Buffer
- RB Buffer
- RW1 Buffer
- RW2 Buffer
  Note: RW2 Buffer contains ethanol. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
- RNase-Free Water
- PALL Nucleic Acid Binding (NAB) Nanosep® Centrifugal Device
- PALL Filtrate Tube of Nanosep® Centrifugal Device

Materials Required but Not Provided

- 1.5 ml microcentrifuge tubes
- Filter tips
- Vortexer
- Microcentrifuge (with the rotor for 1.5 ml tubes)
- ß - Mercaptoethanol (ß - ME)
- DNase I (optional)
- 20-G needle (optional)

Storage and Stability

The kit is stable at room temperature for 1 year from date of receipt.

Total RNA Extraction Kit – *Tissue*

Biomate™

Preparation Before Assay

■ Add 10 µl ß-ME to 1 mL of RL Buffer.

Note: Prepared RL Buffer can be stored at room temperature for up to 1 month.

Sample Preparation

① Cut and place 10 mg of fresh or frozen animal tissue in a RNase-free microcentrifuge tube.

② Add 400 µl of RL Buffer (ß-ME added) into the tube and sufficiently grind the tissue.

Optional: Shear the tissue by passing lysate though a 20-G needle syringe for 10 times.

Note: If insolubles remain after incubation, centrifuge for 2 minutes at 13,000 rpm (10,000 x g) and transfer the clarified supernatant to the new microcentrifuge tube.

Assay Procedures

① Add 400 µl of RB Buffer to the sample and mix by pipetting immediately for 10 seconds.

② Transfer 450 µl of the mixture to Nanosep®.

③ Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes.

④ Remove the retentate cup of Nanosep®, discard the filtrate from the filtrate tube, and then place the retentate cup back into the filtrate tube of Nanosep®.

⑤ Transfer the remaining mixture to Nanosep®.

⑥ Repeat ③ and ④.

Optional: Perform DNA Elimination Procedures between ⑥ and ⑦ if needed.

⑦ Add 450 µl of RW1 Buffer to Nanosep®.

⑧ Centrifuge at 13,000 rpm (10,000 x g) for 1 minute.

⑨ Remove the retentate cup of Nanosep®, discard the filtrate from the filtrate tube, and then place the retentate cup back into the filtrate tube of Nanosep®.

⑩ Add 450 µl of RW2 Buffer to Nanosep®.

⑪ Centrifuge at 13,000 rpm (10,000 x g) for 1 minute.

⑫ Remove the retentate cup of Nanosep®, discard the filtrate from the filtrate tube, and then place the retentate cup back into the filtrate tube of Nanosep®.

⑬ Repeat ⑩, ⑪ and ⑫.

⑭ Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes to dry NAB Filter.

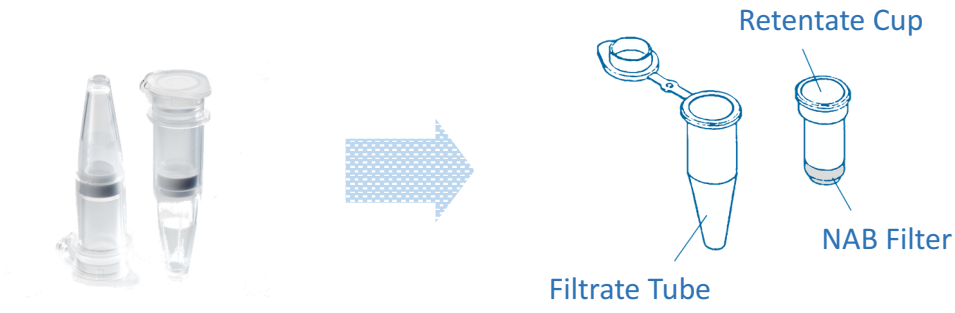
⑮ Remove the retentate cup of Nanosep®, and transfer it into the new filtrate tube.

⑯ Add 50 µl of RNase-Free Water into the CENTER of the retentate cup.

⑰ Let the device stand for at least 2 minutes so NAB Filter can be soaked completely.

⑱ Centrifuge at 13,000 rpm (10,000 x g) for 2 minutes to elute the purified RNA.

PALL Nucleic Acid Binding (NAB) Nanosep® Centrifugal Device



DNA Elimination Procedures (optional)

The contamination of genomic DNA is almost impossible to be avoided during RNA extraction procedures. If the presence of DNA affects downstream applications, DNase I can be used to eliminate the contamination.

The protocol is followed and the procedures shall be performed between ⑥ and ⑦ of RNA extraction.

It is important to ensure DNase applied is highly purified and RNase-free. If RNase is present, even in trace amounts, RNA degradation will be produced.

❶ Add 200 µl of RW1 Buffer to Nanosep®.

❷ Centrifuge at 13,000 rpm (10,000 x g) for 15 seconds.

❸ Remove the retentate cup of Nanosep®, discard the filtrate from the filtrate tube, and then place the retentate cup back into the filtrate tube of Nanosep®.

❹ Prepare DNase I working solution by adding 10 µl of DNase I stock solution (3 Kunitz U/µl) to 70 µl of DNase I Reaction Buffer, mixing by gently inverting the tube, and centrifuge briefly to collect residual liquid from the sides of the tube.

Note: DNase I is especially sensitive to physical denaturation. Mixing should only be carried out by gently inverting the tube. Do not vortex.

❺ Add all DNase I working solution (80 µl) to Nanosep®.

❻ Incubate at 20 – 30 °C for 15 minutes.

❼ Repeat ❶, ❷, ❸.

Troubleshooting Guide

- **DNA contamination**
  Perform DNA Elimination Procedures.
- **Eluted RNA does not perform well in downstream applications**
  Contamination of ethanol residue.
  To solve the problem, dry NAB Filter of Nanosep® with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes after washing steps.
- **Low RNA Yield**
  - Ensure the bottle of RW2 Buffer closed tightly after each use to avoid ethanol evaporation.
  - Insufficient homogenization/too much starting material, please adjust it.
  - RNA still bound to NAB Filter of Nanosep®. Elute twice to increase the yield.
  - Contamination of ethanol residue. Dry NAB Filter of Nanosep® with additional centrifugation at 13,000 rpm (10,000 x g) for 5 minutes after washing steps.
  - Ensure RNase-Free Water is added into the CENTER of the retentate cup.
- **RNA Degradation**
  - Ensure tissue samples were stabilized immediately after harvest, and were prepared according to the protocol.
  - Avoid RNase contamination by wearing gloves and masks throughout the whole process, and ensuring all materials applied RNase-free.