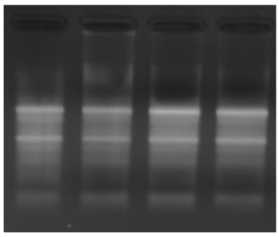
Total RNA Extraction Kit – *Blood & Cell*

Cat. No. **RB10**

For total RNA extraction from Whole Blood & Cultured Cells

***Biomate™ Total RNA Extraction Kit – Blood & Cell** is designed by patented technology for purifying total RNA from bacterial, cultured cells and fresh human whole blood. 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.*

1 2 3 4

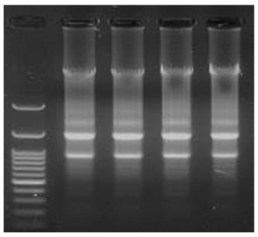


Total RNA extracted from Whole Blood

Total RNA from 200 µl of whole blood samples was extracted by Biomate™ ***Total RNA Extraction Kit – Blood & Cell*** or the kit from ***Competitor Q***. 10 µl of 60 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1% agarose gel.

1-2: Competitor Q
3-4: Biomate™

M 1 2 3 4



Total RNA extracted from Cells

Total RNA from 1 x 10^6 HeLa Cell samples was extracted by Biomate™ ***Total RNA Extraction Kit – Blood & Cell*** or the kit from ***Competitor Q***. 10 µl of 60 µl eluates of purified Total RNA was analyzed by electrophoresis on a 1% agarose gel.

1-2: Competitor Q
3-4: Biomate™
M: Marker

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

- RBC Lysis Buffer
- 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 (for cell samples) or ≥ 3 ml (for blood samples) microcentrifuge tubes
- Filter tips
- Vortexer
- Microcentrifuge
- ß - Mercaptoethanol (ß - ME)
- DNase I (optional)

Storage and Stability

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

Total RNA Extraction Kit – *Blood & Cell*

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.

【Whole Blood】
Sample Preparation

① Add 400 µl of human whole blood with 2 ml of RBC Lysis Buffer in a ≥ 3ml RNase-free microcentrifuge tube, and mix completely by inversion. Do not vortex.
Note: For optimal results, the volume of the mixture (blood + RBC Lysis Buffer) should not exceed 4/5 of the volume of the tube to allow efficient mixing.

② Incubate on ice for 10 minutes and invert 3 times during incubation.

③ Centrifuge at 4°C at 2,500 rpm (500 x g) for 5 minutes, and discard the supernatant completely.

④ Add 400 µl of RBC Lysis Buffer to the cell pellet. Resuspend cells by vortex briefly.

⑤ Incubate on ice for 3 minutes.

⑥ Centrifuge at 4°C at 2,500 rpm (500 x g) for 5 minutes, and discard the supernatant completely.

⑦ Add 400 µl of RL Buffer (ß-ME added) to the cell pellet and mix by vortexing.

⑧ Incubate at room temperature for 5 minutes.

【Cell】
Sample Preparation

① Transfer the max. 5 x 10^6 of cells to a microcentrifuge tube.

② Centrifuge at 4°C at 2,500 rpm (500 x g) for 5 minutes, and discard the supernatant completely.

③ Add 400 µl of RL Buffer (ß-ME added) and mix by vortexing.

④ Incubate at room temperature for 5 minutes.

Assay Procedures

PALL
Nucleic Acid Binding (NAB)
Nanosep® Centrifugal Device

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.

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.