EcoSpin Blood Total RNA Kit

50 rxns

Cat No: E2090

Shipping : Ship at ambient temperature.
Storage : Store the kit between 15°C and 25°C.
Store *EcoSpin* RBCL Buffer at 4-8°C upon receipt.

General Information

EcoSpin Blood Total RNA Kit is designed as a simple and convenient purification of high-quality total RNA including small RNAs (e.g. microRNAs) from whole blood up to 8 ml. This kit utilizes chaotropic ions and silica-based membrane technology, eliminating the need for expensive resins, hazardous phenol-chloroform extractions, β-mercaptoethanol, or time-consuming alcohol precipitation. The standard protocol lasts less than 40 minutes at room temperature and purified RNA can be effectively used in routine downstream applications including cDNA synthesis, northern blotting, differential display, primer extension, and mRNA selection.

Kit Contents

EcoSpin RBCL Buffer	(250 ml concentrate)
EcoSpin Lysis/Binding Buffer	(22 ml)
EcoSpin Wash Buffer 1	(22 ml)
EcoSpin Wash Buffer 2*	(8 ml concentrate)
EcoSpin Elution Buffer	(5 ml)
EcoSpin Columns	(50)
EcoSpin Collection Tubes	(50)

*Add 32 ml absolute ethanol

Protocol for Whole Blood Total RNA

Each isolation procedure is suitable for isolation of total RNA from up to 8 ml of non-coagulating fresh whole blood collected using EDTA as the anti-coagulant.

Important: It is recommended to dilute the *EcoSpin* RBCL Buffer and warm the 1x working solution to room temperature prior to use.

Note that the blood should be fresh and at room temperature. Blood samples should have been collected in a tube containing EDTA or any other anticoagulant.

Step 1. Collection of mono-nuclear cells

1. Dilute the *EcoSpin* RBCL Buffer to a 1x working solution by adding 1 part *EcoSpin* RBCL Buffer to 9 parts room temperature RNAse free water. Warm the solution to room temperature.

2. Transfer the fresh whole blood sample into a 50 ml RNase-free falcon tube (not provided). Add diluted 1x *EcoSpin* RBCL Buffer at a ratio of approximately 1 part blood to 6 parts lysis buffer.

3. Invert the tube to mix and incubate for 10 minutes at room temperature protected from light. Invert the sample at regular intervals by hand. Do not vortex.

4. Centrifuge the tube at 1500 rpm for 5 minutes. Carefully remove and discard the clear, red supernatant without disturbing the cell pellet.

5. Gently resuspend the pellet in 1 ml of diluted 1x *EcoSpin* RBCL Buffer and transfer to a RNase-free 1.5 ml microcentrifuge tube (not provided). Do not vortex.

6. Incubate for 5 minutes at room temperature protected from light.

7. Centrifuge the tube at 1500 rpm for 3 minutes. Carefully remove and discard the supernatant without disturbing the cell pellet.

8. Gently resuspend the pellet in 1 ml of Phosphate Buffered Saline (not provided) and pellet cells as in step 7. Carefully remove and discard the supernatant without disturbing the cell pellet.

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Step2. RNA Isolation

9. Add 400 µl *EcoSpin* Lysis/Binding Buffer and mix well by pipetting. Homogenize the lysate using a syringe and needle by passing the lysate through a 20-gauge (0.9 mm) needle, attached to a sterile plastic syringe, at least 5–10 times or until a homogeneous lysate is achieved.

Precaution: *EcoSpin* Lysis/Binding Buffer has the ability to inactivate the RNases in cells, however, 4 µl β-mercaptoethanol might be added to each 400 µl *EcoSpin* Lysis/Binding Buffer to ensure complete inactivation of RNases.

10. Add 400 µl absolute (96-100%) ethanol to the lysate and mix well by vortexing for 10 seconds.

11. Insert an *EcoSpin* Column into a Collection Tube and transfer 700 µl sample from step 3 to the *EcoSpin* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature. Depending on your lysate volume, repeat Step 4 as necessary.

Optional: *EcoSpin* Blood Total RNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

12. Discard the flow through and add 400 µl *EcoSpin* Wash Buffer 1 to the *EcoSpin* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

13. Discard the flow through and add 500 µl *EcoSpin* Wash Buffer 2 to the *EcoSpin* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

14. Discard the flow through and add 200 µl *EcoSpin* Wash Buffer 2 to *EcoSpin* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

15. Transfer the *EcoSpin* Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).

16. Add 30-50 µL of *EcoSpin* Elution Buffer to the center of the *EcoSpin* Column membrane and incubate the column at room temperature for 1 minute.

17. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

18. Discard the *EcoSpin* Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.

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