EcoSpin FFPE Total RNA Kit

50 rxns

Cat No: E2095

Shipping : Ship at ambient temperature.
Storage : Store the kit between 15°C and 25°C. Store Proteinase K at -20°C.

General Information

EcoSpin FFPE Total RNA Kit is designed as a simple and convenient purification of total RNA from formalin-fixed, paraffin-embedded (FFPE) tissue materials. 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.

Kit Contents

EcoSpin Pre-Lysis Buffer	(8 ml)
EcoSpin FFPE Lysis Buffer	(8 ml)
EcoSpin Binding Buffer	(22 ml)
EcoSpin Wash Buffer 1	(22 ml)
EcoSpin Wash Buffer 2*	(10 ml concentrate)
EcoSpin Elution Buffer	(5 ml)
EcoSpin Proteinase K#	(lyophilized)
EcoSpin Columns	(50)
EcoSpin Collection Tubes	(50)

*Add 40 ml absolute ethanol

Reconstitute lyophilized Proteinase K in 1.1 ml Proteinase K Storage Buffer. Proteinase K solution is stable for 1 year when stored at 4°C. For long-term storage (>1 year) store Proteinase K solution at -20°C.

Protocol for FFPE Total RNA

Each isolation procedure is suitable for isolation of total RNA from up to 5 freshly cut sections of up to 20 µm thick from the interior of an FFPE tissue block. A total of up to 250 mm^2 surface area is recommended as starting material. If extraction of total RNA from more sections is required, scale up the amounts of reagents used in the entire protocol proportionally.

1. Transfer the sections into an RNase-free microcentrifuge tube. Remove any excess paraffin.

2. Add 1 mL of xylene to the sample. Incubate at 50°C for 5 minutes. Mix by vortexing.

3. Centrifuge at maximum speed for 2 minutes.

4. Carefully remove the xylene completely.

5. Add 1 mL of 96 - 100% ethanol. Mix by vortexing.

6. Centrifuge at maximum speed for 2 minutes and discard the supernatant.

7. Repeat steps 5-6.

8. Air dry the pellet for about 10 minutes at room temperature to completely remove the residual ethanol.

9. Add 100 µl *EcoSpin* Pre-Lysis Buffer and mix well by vortexing or pipetting up and down.

10. Add 100 µl *EcoSpin* FFPE Lysis Buffer and mix thoroughly. Add 20 µl *EcoSpin* Proteinase K and mix well. Incubate at 55°C for 15 min with frequent vortexing.

Note: Extending incubation time will not increase the yield.

11. Incubate at 80°C for 15 min with frequent vortexing.

12. Centrifuge at maximum speed for 1 minute and discard the supernatant.

13. Add 400 µl *EcoSpin* Binding Buffer and mix well.

14. Add 600 µl absolute (96-100%) ethanol and mix well by vortexing for 10 seconds.

15. Insert an *EcoSpin* Column into a Collection Tube and transfer sample from step 14 to the *EcoSpin* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 sec at room temperature. Depending on your lysate volume, repeat Step 14 as necessary.

Optional: *EcoSpin* FFPE 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.

16. 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.

17. 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.

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18. 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.

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

20. Add 50-100 µL of *EcoSpin* Elution Buffer to the center of the *EcoSpin* Column membrane and incubate the column at room temperature for 1 minute.

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

22. 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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