

EZ-10 Spin Column Blood RNA Mini-Preps Kit



PRODUCT INFORMATION

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BS82313 Kit Contents

Components	BS82313, 50 Preps
Buffer Rlysis-RG	12 ml
Buffer NS-A	22.5 ml
2% SDS	2.5 ml
Universal GT Solution*	18 ml
Universal NT Solution*	6 ml
RNase-free Water	30 ml
EZ-10 Spin Column	50
2 ml Collection Tube	50
Protocol	1

*Universal GT Solution and Universal NT Solution are supplied in a concentrated form, before use; add 12 ml 96-100% ethanol to 18 ml concentrated **universal GT solution** and 24 ml 96-100% ethanol to 6 ml concentrated **universal NT solution** to make a work solution.

NOTE: Care must be taken when working with RNA. It is important to maintain an RNase-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNase free tubes, tips, gels. Wear gloves at all times.

Introduction

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EZ-10 Column Blood RNA Purification Kit provides a simple spin column technique for preparation of high quality, high-purity intact total RNA. The reagent contains disruptive and protective properties of guanidine isothiocyanate and β -mercaptoethanol to inactivate the ribonucleases present in cell extracts. RNA in the whole homogeneity is selectively absorbed on spin column and other impurities are washed away. Total RNA is eluted from the membrane in the presence of RNase-free water.

5-15 μ g total RNA can be purified from 200 μ l of anticoagulated blood sample using this kit. Purified RNA is ready for most downstream applications such as RT-PCR, Northern Blotting, Poly (A) selection and in vitro translation.

Features

- ü Fast. Using a rapid spin-column format, the entire procedure takes approx 15 minutes.
- ü High Purity of RNA. OD_{260}/OD_{280} ratio of purified RNA is generally > 1.9 .
- ü Compatible with downstream applications such as Northern Blots, cDNA synthesis, RT-PCR and qRT-PCR.
- ü High Quality RNA. Buffer Rlysis-AG maintains the integrity of the RNA, no degradation.
- ü Economic.

Materials Supplied by User:

Microcentrifuge capable of at least 12,000 $\times g$
RNase-Free pipets and pipet tips
Vortexer
RNase-Free Ethanol (96-100%)
RNase-Free Microcentrifuge tubes (1.5 ml or 2 ml)

Procedures

1. Add 0.1-0.3 ml fresh anticoagulated whole blood to a 1.5 ml RNase-free centrifuge tube. Add 0.5 ml RNase-free Water and mix by inverting.
2. Centrifuge at 8,000 $\times g$ for 1 minute at room temperature,

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discard the supernatant (plasma).

3. Using RNase-free pipet tips, add 200 μ l Buffer Rlysis-RG and mix by inverting immediately.
4. Incubate at room temperature for 5 minutes to make sure the cells are completely lysed.
5. Add 360 μ l Buffer NS-A, 40 μ l of 2% SDS and mix by inverting the tube several times.

Note. there may be precipitates after addition of SDS. Proceed to step 6 as precipitates will not affect performance of the kit.

6. Centrifuge at 12,000 $\times g$ for 5 minutes at 4°C. Transfer the supernatant to a new RNase-free 1.5 ml tube.
7. Add 1/2 volume of ethanol, mix by inverting the tube.
8. Transfer the solution to the spin column, centrifuge at 12,000 $\times g$ for 1 min at room temperature, discard the flow-through.
9. Add 0.5 ml of Universal GT Solution to the column, centrifuge at 12,000 $\times g$ for 1 min at room temperature, discard the flow-through.
10. Add 0.5 ml of Universal NT Solution to the column, centrifuge at 12,000 $\times g$ for 1 min at room temperature, discard the flow-through.
11. Centrifuge the column at 12,000 $\times g$ for additional 1 min at room temperature.

Note: This step is very important to remove the residual ethanol thoroughly.

12. Place the column in a new RNase-free 1.5 ml centrifuge tube, add 50 μ l RNase-free Water. Keep at room temperature for 2 minutes. Centrifuge at 12,000 $\times g$ for 30 sec at room temperature, store RNA solution at -80°C.