RUO

Cat.No.		
	G2MBR4-0581	50 T
	G2MBR4-0582	250 T

SPINXT Blood RNA Extraction kit

INTENDED USE.

The SpiNXT Blood RNA Extraction kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products.

INTRODUCTION

SpiNXT Blood RNA Extraction kit provides a cost effective and a convenient way for the purification of total RNA from fresh whole blood. The kit makes use of silica based spin column technology which allows complete removal of inhibitors such as divalent cations and proteins. The columns have a high binding capacity and high quality RNA is obtained from whole blood. The optimized high-salt buffering system allows RNA to bind to the silica matrix of the spin column while contaminants pass through the column. Impurities are efficiently washed away, and the pure RNA is eluted in the Buffer AE provided with the kit. The RNA obtained is compatible with various routine applications such as, RT-qPCR, Northern blotting and other RNA based analysis.

KIT CONTENTS

Kit Contents	Kit Content Code - 50T	Kit Content Qty.	Kit Content Code - 250T	Kit Content Qty.
Buffer LBR	G2MBR3-1273-2	1 X 25ml	G2MBR3-1273-1	1 X 110ml
Buffer RW1	G2MBR3-1274-2	1 X 16ml	G2MBR3-1274-1	1 X 60ml
Buffer RW2	G2MBR3-1275-2	1 X 20ml	G2MBR3-1275-1	2X40ml
Buffer AE	G2MBR3-1276-2	1 X 5ml	G2MBR3-1276-1	1 X 15ml
Proteinase K	G2MBR3-1277-2	1 X 24mg	G2MBR3-1277-1	1 X 110mg
Protease Dissolve Buffer	G2MBR3-1278-2	1 X 1.5ml	G2MBR3-1278-1	1 X 6ml

Consumable

Item Name	Kit Content Qty for 50 T	Kit Content Qty for 250 T
Mini columns	1 x 50 Nos.	2 x 125 Nos.
Collection Tubes	1 x 50 Nos.	2 x 125 Nos.

For research use only.

STORAGE AND STABILITY

This product can be stored at room temperature (15-25°C) for 12 months. If precipitate forms in any of the reagents of the kit, warm at 55ºC to dissolve.

INSTRUCTIONS BEFORE USE

- Preheat a water bath or heating block to 56°C.
- Dilute Buffer RW1 & RW2 with an appropriate amount of ethanol (96-100%) as shown on label and store at room temperature.
- Add Protease Dissolve buffer to the Proteinase K final concentration should be 20mg/ml. For storage, the unused portion of the solution may be stored in aliquots at -20°C until needed.
- Re-suspend any precipitate by warming it at 55 ºC and let it stay at room temperature so that it cools back to 25 ºC before using.
- All purification steps needs to be carried out at room temperature (15-25 ºC).
- The purified RNA has A260/280 ratio between 1.9 to 2.1, however, when RNA concentration is lower than 20ng/µl, deviation from the expected ratio will be observed.

RNA HANDLING AND STORAGE

- Purification of RNA should be achieved on the same day on which sample collection has been done.
- Do not freeze the blood samples.
- Keep the RNA on ice after extraction and while working with it.
- All steps must be carried at RTexcept centrifugation.
- Store the extracted RNA at -80°C.

RNA CONTAMINATION

RNA purity and integrity is very essential for downstream applications.RNaseA usually degrades the RNA, which is a very common contaminant found in any lab environment. Care must be taken that no RNases comes in contact while RNA preparation specially during washing steps through the columns or elution steps. General recommendations include.

- Wear gloves while handling reagents and at all times during the preparation.
- Change gloves frequently.
- Use sterile, disposable RNase free pipette tips.
- Clean the working area and non-disposable items (pipettes & centrifuges) with reagents which are known to remove RNase contamination.
- Keep all the kit components tightly sealed when not in use. Cap the bottles immediately after use.

Important Notes

Sample collection, storage and handling

BLOOD

SpiNXT Blood RNA Extraction kit provides the purification of total cellular RNA from fresh, human whole blood. Whole blood should be collected in the EDTA, Heparin or Citrate coated vial. For maximum results, sample should be processed immediately or after few hours of collection.

Blood samples should not be stored for longer periods before the isolation of RNA

PROTOCOL

A. Whole Blood RNA Extraction protocol

1) Transfer 400 µl of fresh whole blood along with 400µl Buffer LBR in a fresh 2ml micro-centrifuge tube.
2) Then further add 20 µl of Proteinase K into the solution.
3) Incubate the micro-centrifuge tube for 10 minutes at room temperature (Thermo shaker at RT and 1100 rpm can also be used for efficient lysis).
4) Further, add 400 µl 70% chilled ethanol into the sample. Invert mix and incubate it at room temperature for 1 minute.
5) Transfer whole mixture to the spin column and centrifuge at 12,000xg for 30sec at 4°C.
6) Discard the collection tube with the flow through.
7) Place the mini column into a fresh collection tube and add 500 µl of RW1 to the center of the column without wetting the rim. Centrifuge it at 10,000xg for 30 Sec at 4°C. Discard the flow through.
8) Place the mini column back into the collection tube (2ml) and add 700 µl RW2, 10,000xg for 30 Sec at 4°C. Discard the flow through.
9) Repeat the step 8.
10) Dry spin: Centrifuge the column one more time at 20,000xg for 2 minutes.
11) Transfer the column into a fresh 1.5 ml micro-centrifuge tube. Add 30-50 µl of Buffer AE into the center of the silica membrane and kept for 3-5 minutes on ice (preferable).
12) Centrifuge at 10,000xg for 1 minutes.
13) Store the eluted RNA at -20 degree celsius and for long term storage -80 degree Celsius is preferable.

Symbols for use in the labeling	
Symbols	**Definition**
	KEEP AWAY FROM SUNLIGHT
	TEMPERATURE LIMIT
	RESEARCH USE ONLY
	UPWARD
	CONSULT INSTRUCTIONS FOR USE
	BATCH CODE
	CATALOGUE NUMBER
	USE BY DATE
	DATE OF MANUFACTURE
	MANUFACTURER
	CONTAINS SUFFICIENT FOR <N> TESTS
	CAUTION
	DO NOT USE IF PACKAGE IS DAMAGED

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