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Column Based



















SN BLOOD RNA EXTRACTION KIT

(Column Based)

DESCRIPTION:

SN Blood RNA Extraction kit provides a cost- effective system designed for the fast and easy isolation of the RNA fragments from whole blood. The preparation is based on a silica-membrane technology, of binding RNA in high-salt and elution in low-salt buffer.

CONTENTS OF KIT:

Sl.	Components	Volume		
No		5 Rxn	50 Rxn	100 Rxn
1	LS Buffer	1.5ml	15ml	30ml
2	BB Buffer	2ml	20ml	40ml
3	W1 Buffer	1.6ml	16ml	32ml
4	Elution Buffer	250µl	2.5ml	5ml
5	SN Spin Column & Collection tube	5 No.	50 No.	100 No.

NOTE: Preparation for first use after receiving the kit (Add 100% Ethanol to Wash Buffer) (5Rxn: 3.4ml, 50Rxn: 34ml, and 100Rxn: 68ml) mix well and store the buffer at room temperature.

- If Proteinase K shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:100µl,50Rxn: 1ml, 100Rxn: 2ml)
- If DNase and Carrier -RNA shipped in lyophilized form, upon receiving resuspend with Nuclease free water (5Rxn:50μl, 50Rxn:500μl, 100Rxn: 1ml)
- Store Proteinase k/ DNase/ Carrier-RNA at
 -20°C either liquid form (or) Lyophilized form

REQUIRED MATERIALS BUT NOT PROVIDED:

- ✓ 100% Ethanol
- ✓ Dry bath
- ✓ 1.5ml Centrifuge tubes
- ✓ micro centrifuge
- ✓ Vortex

STORAGE / SHIPPING:

- Shipped at: Ambient Temperature.
- Storage: All Buffers stored at Room temperature...

SPECIFICATIONS:

Principle	Spin Column

Binding Capacity: ~30-40μg genomic RNA

Recommended input amount : $200 \, \mu I$ whole blood

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Elution Volume: 50µl

Purity: A260/280 - 1.8±0.1, A260/230 - 2.0±0.1

Compatible Downstream Applications : Endpoint PCR, qPCR, Sequencing, etc.

Expected Yield : Up to $10 \mu g$ (depending upon the type. Quality & quantity of the starting material used)

PROCEDURE:

- 1) Take 200µl blood sample in a 1.5ml centrifuge tube.
- 2) Add 300μl LS Buffer, 20μl Proteinase K and 10μl of DNase and 10μl of Carrier-RNA. Resuspend by gentle pipetting and vortex for 30secs.
- Incubate at 56°C for 15mins. Then add 400µl of BB Buffer, resuspend by gentle pipetting and vortex thoroughly for 30secs.
- 4) Transfer entire lysate to SN Spin Column and leave at room temperature for 2mins.
- 5) Centrifuge for 5mins at 15,000rpm and discard flow through.
- 6) Add 500µl of W1 buffer and centrifuge for 2mins at 15,000rpm and discard flow through.
- 7) Repeat step (6)
- 8) Centrifuge at 15,000rpm for an additional 1min to dry the column.
- 9) Place the SN Spin Column in a new 1.5ml centrifuge tube and incubate for 10mins at 56°C. (with cap open). This step is very important to remove the residual ethanol.

NOTE: Elution should be pre-heated for 10mins before adding into the SN Spin Column.

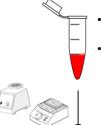
 Add 50μl of pre-heated Elution to the membrane center of SN Spin Column and incubate for 4mins at 56°C (with cap close).

Important step: For effective elution, make sure that the elution buffer is dispensed onto the membrane center and is absorbed completely.

10) Centrifuge the tube at 15,000rpm for 1 min to Elute the RNA (store at -20°C).

Important: Do not elute the RNA using less than suggested volume. It will lower the yield.

FLOW CHART:



- Take 200µl of Blood sample in a 1.5ml centrifuge tube.
- Add 300µl of LS Buffer, 20µl Proteinase K, 10µl of DNase and 10µl of carrier -RNA to it.
 - Mix well by gentle pipetting and vortex for 30secs.
 - Incubate at 56°C for 15mins
 - Add 400µl BB Buffer
 - Mix well by gentle pipetting
 - Vortex thoroughly for 30secs



Transfer entire lysate to SN Spin Column and leave 2mins at room temperture



Centrifuge for 5mins at 15,000 rpm and discard flow through.



Add 500µl W1 buffer



- Centrifuge for 2mins at 15,000 rpm
- Discard flow through



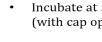
- Add 500µl W1 buffer Centrifuge for 2mins at 15,000 rpm.
- Discard flow through.



Centrifuge additional 1min at 15,000 rpm.



Place the SN Spin Column in a new 1.5ml Eppendorf tube



- Incubate at 56°C for 10mins (with cap open)
- Add 50µl of pre-heated Elution Buffer to the membrane center of SN Spin Column (with close cap)
- Incubate at 56°C for 4mins
- Centrifuge for 1min at 15,000
- Elute Pure RNA (store at -20°C)

TROUBLE SHOOTING:

Problems	Possible	Solutions
	reasons	
Low or none recovery of RNA fragment	Apply more than 200µl Blood cells	If product is more than 200µl, separate it into multiple tubes.
	Elution of RNA fragment is not efficient	Make sure the pH of Elution Buffer or ddH ₂ 0 is between 7.5-8.0. Make sure that the elution solution has been completely
		absorbed by the column membrane before centrifugation.
		Preheat the elution solution to 56°C before use.
Poor Performa- nce in the	Salt residue remains in eluted RNA	Wash the column twice with W1 Buffer.
downstre am application s	Ethanol residue remains in eluted RNA	Do discard the flow- through after washing with W1 Buffer and centrifuge for an additional 1min.

IMPORTANT NOTES:

- a) Buffer provided in this kit contains irritants. Wear gloves and lab coat when handling these buffers.
- b) Add required volume of ethanol (96-100%) to wash Buffer before use.
- c) Centrifugation steps are done by a micro centrifuge capable of the speed at 15,000 rpm.
- d) Fresh TBE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.
- Then excising the agarose gel, make sure to shorten the time of exposure to ultraviolet irradiation.