SHANKARANARAYANA LIFE SCIENCES
A NEW GENERATION COMAPANY

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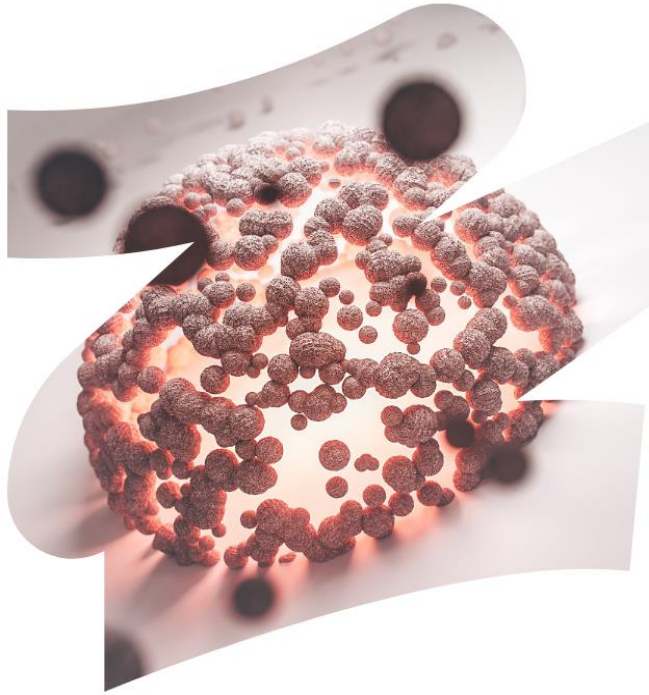
SnLs
A NEW GENERATION COMPANY



SN Blood RNA extraction Kit

Magnetic Bead-Based

Innovation Speaks

SN BLOOD RNA EXTRACTION KIT

(Magnetic Bead - Based)

DESCRIPTION:

SN Blood RNA Extraction Kit provides an accurate, easy to use & rapid method to isolate high quality of RNA from various blood samples. It does not contain harmful organic compounds such as phenol and chloroform. The preparation is based on a Magnetic Bead-Based technology for binding RNA in high-salt and elution in low-salt buffer.

CONTENTS OF KIT:

Sl. No	Components	Volume 5 Rxn	50 Rxn	100 Rxn
1	LS Buffer	1ml	10ml	20ml
2	BB Buffer	1.5ml	15ml	30ml
3	W1 Buffer	1.5ml	15ml	30ml
4	Elution Buffer	250µl	2.5ml	5ml
5	Magnetic Bead	250µl	2.5ml	5ml

- **NOTE: Preparation for first use after receiving the Kit: Add 100% Ethanol to Wash Buffer (5Rxn: 1ml, 50Rxn:10ml, and 100Rxn: 20ml)** 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:2)**
- If **DNase** shipped in lyophilized form, upon receiving resuspend with Nuclease free water (**5Rxn:50µl,50Rxn:500µl,100Rxn:1ml**
- **Proteinase k/DNase are store at -20°C either in liquid form or Lyophilized form.**

REQUIRED MATERIALS NOT PROVIDED

- ✓ 100% Ethanol (CHILLED)
- ✓ Dry bath
- ✓ 1.5ml Centrifuge tubes
- ✓ micro centrifuge
- ✓ Vortex
- ✓ Magnetic rack

STORAGE / SHIPPING:

- Shipped at: Ambient Temperature.
- Storage: All buffers can be stored at room temperature.

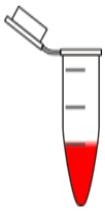
SPECIFICATIONS:

Principle	Mag-Bead
Recommended Input Amount : 200µL sample	
Binding Capacity : ~20-30µg RNA	
Elution Volume : 50µl	
Purity: A260/280 - 1.8±0.1, A260/230 - 2.0±0.1	
Compatible Downstream Applications : PCR, Cloning, Next generation sequencing etc.	
Expected Yield : depending upon the type	

PROCEDURE:

1) Take 200µl of blood sample in a 1.5ml centrifuge tube and add 200µl of LS Buffer, 20µl of Proteinase K ,10µl of DNase mix by gentle pipetting and vortex for 30secs.
2) Incubate at 56˚C for 10mins. Add 200µl of chilled 100% ethanol to it and leave at room temperature for 1min.
3) Then add 300µl of BB Buffer along with 50µl of SN Magnetic bead and mix well by vortexing for 30secs, keep at room temperature for 10mins.
4) Place the tube upon the magnetic rack (let the bead attracts towards magnet). Then remove the supernatant without disturbing the bead.
5) Add 500µl of W1 buffer, mix well by vortexing for 30secs and keep at room temperature for 10mins.
   NOTE:(W1 buffer concentration per reaction: W1 buffer -300µl: 100% ETOH -200µl)
6) Place the tube upon the magnetic rack (let the bead attracts towards magnet). Then remove the supernatant without disturbing the bead .
7) Add 500µl of 70% ethanol, mix well by vortexing for 30secs and keep at room temperature for 5mins.
8) **REPEAT THE STEP (6)**
9) Incubate the centrifuge tube at 56˚C for 10mins (with cap open).
   NOTE: Elution should be pre-heated for 10mins before adding into the tube.
10) Add 50µl of pre-heated Elution to the tube vortex for 10secs and then incubate at 56˚C for 4mins (with close cap)
11) Vortex for 10secs and centrifuge the tube at 15,000rpm for 2mins to Elute pure RNA. (Store at -20°C)

FLOW CHART:

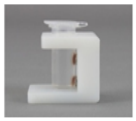


- Add 200µl Blood , 200µl of LS Buffer ,20µl Proteinase K and 10µl of DNase in a tube
- Mix well by gentle pipetting and pulse-vortexing for 30secs.
- Incubate at 56˚C for 10mins. Add 200µl of 100% ethanol and leave for 1min.

- Add 300µl of BB Buffer along with 50µl of SN magnetic bead. Vortex thoroughly for 30secs
- Then keep the tube at room temperature for 10mins

- Place the tube upon the magnetic rack, it attracts bead and separates.
- Discard the supernatant without distrubing bead

- Add 500µl W1 buffer by gentle pipetting ,vortex for 30secs and allow to stand for 10mins at Room temperature.



- Place the tube upon the magnetic rack, it attracts bead and separates. Discard the supernatant without distrubing bead.
- Add 500µl of 70% ethanol and vortex for 30secs then allow to stand for 5mins at Room temperature.
- Place the tube upon the magnetic rack, it attracts bead and separates.
- Discard the supernatant without distrubing bead.

- Incubate at 56˚C for 10mins for ethanol evaporation (with cap open)

- Add 50µl of pre-heated Elution Buffer to the tube vortex for 10secs (with cap closed)
- Incubate at 56˚C for 4mins. Vortex for 10secs.
- Centrifuge for 2mins at 15,000 rpm

- Elute Pure RNA **(Store at -20°C)**

TROUBLE SHOOTING:

Problems	Possible reasons	Solutions
Low or none recovery of RNA fragment	Apply more than 200µl Sample.	If product is more than 100µl, separate it into multiple tubes.
	Elution of RNA fragment is not efficient	Make sure the pH of Elution Buffer is between 7.5- 8.0.
		Make sure that the elution solution has been observed by the magnetic beads.
Poor Performance in the down stream applications	Salt residue remains in eluted RNA	Preheat the elution solution at 56°C before use.
	Ethanol residue remains in eluted RNA	washing step is done with W1 Buffer and 70% ethanol .

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) Fresh TBE electrophoresis buffer is recommended on electrophoresis for experiments requiring high purity.
d) Then excising the agarose gel, make sure to shorten the time of exposure to ultraviolet irradiation.