HELINI
Purefast
Human Blood RNA
Mini spin prep kit

Instructions for use

For use with: Fresh human whole blood

CE
IVD

REF 2011

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HELINI Biomolecules, Chennai, INDIA

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Contents

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Intended Use

HELINI Purefast Blood RNA Mini spin prep Kit is designed for rapid and cost-effective small-scale preparation of high-quality total RNA from fresh, whole human blood. Whole blood should be collected in the presence of an anticoagulant, preferably EDTA, although other anticoagulants such as citrate, heparin, or ACD (acid citrate dextrose) can also be used. For optimal results, blood samples should be processed within a few hours of collection. Purified viral RNA can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests
10X RBC lysis buffer	3ml	75ml	150ml	2 x 150ml
Lysis buffer	560µl	14ml	28ml	56ml
Elution Buffer	60µl	2.5ml	5ml	10ml
Wash Buffer-1*	500µl	9 ml	18ml	36ml
Wash Buffer-2*	2x500µl	6 ml	12ml	24ml
Spin columns with collection tube	1	25	50	100
Collection tubes	5	125	250	500

***Wash buffers supplied as a concentrate. Working buffers needs to prepare before use. Please refer page.9**

Storage

- The kit is shipped in room temperature.
- Kit consumables store at room temperature.
- They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.

Material and instruments required

- Ethanol [96 – 100%]
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.]

Product Use Limitations

- All reagents may exclusively be used in molecular biology DNA/RNA applications.
- The product is to be used by personnel specially instructed and trained in Molecular biology experiments.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Discard sample and assay waste according to your local safety regulations.

Technical Assistance

For technical assistance and more information, please contact;

0091-9382810333
0091-44-244490433
helinibiomolecules@gmail.com

Wash buffers - Preparation

Add the indicated volume of ethanol (96-100%) to Wash Buffer I (concentrated) and Wash Buffer II (concentrated) prior to first use:

	Cat.No:2011– 25 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	9ml	6ml
Ethanol [96 – 100%] to add	6ml	24ml
Total volume	**15ml**	**30ml**

	Cat.No:2011 – 50 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	18ml	12ml
Ethanol [96 – 100%] to add	12ml	48ml
Total volume	**30ml**	**60ml**

	Cat.No:2011 – 100 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	36ml	24ml
Ethanol [96 – 100%] to add	24ml	96ml
Total volume	**60ml**	**120ml**

Important Notes:

All purification steps should be carried out at room temperature.

All centrifugations should be carried out in a table-top micro-centrifuge at >12000 x g (12000-14000 rpm, depending on the rotor type).

Adjustment of sample volume:

If your sample volume is less than 200µl, the sample volume should be adjusted with PBS.

If sample volume to be used more, Scale up buffers volume accordingly.

For optimal results, blood samples should be processed within a few hours of collection. Prepare required volume of 1X RBC lysis buffer.

Procedure:

1. Transfer 1.5ml of whole human blood into 15ml centrifuge tube. Add 8.5ml of 1X RBC lysis buffer. Gently mix by vortex well and incubate in ice for 10min. Mix by vortex briefly 2 times during incubation.

2. Centrifuge at 6000rpm for 10min at 4ºC and discard the supernatant carefully.

3. Add 5ml of 1X RBC lysis buffer to the pellet and gently vortex to dislodge the pellet. Incubate in ice for 5min.

4. Centrifuge at 6000rpm for 10min at 4ºC and discard the supernatant carefully.

5. Add 5ml of 1X RBC lysis buffer to the pellet and gently vortex to dislodge the pellet. Incubate in ice for 2min.

6. Centrifuge at 6000rpm for 10min at 4ºC and discard the supernatant carefully.

7. Add 0.15ml of sterile distilled water to the pellet and gently vortex to dislodge the pellet. Transfer the mix in to fresh 1.5ml centrifuge tube.

8. Add 550µl of Lysis Buffer. Mix well by pulse vortexing thoroughly. Centrifuge few seconds to bring down drops to the bottom of the tube.

9. Incubate in room temperature for 5min.

10. Add 550µl of [100%] ethanol and mix well by vortexing for 30seconds. Spin down few seconds to bring down drops to bottom of the tube.

11. Transfer 620µl into the Purefast® spin column. Centrifuge at 8000rpm for 1 min. Discard the flow-through with collection tube and place the spin column into the fresh 2ml collection tube.

12. Transfer remaining 620µl/entire lysate in to the same Purefast spin column. Centrifuge at 8000rpm for 1 min. Discard the flow-through with collection tube and place the spin column into the fresh 2ml collection tube.

13. Add 500µl of Wash buffer-1 [Ethanol added] to the Purefast® spin column. Centrifuge at 8000rpm for 1min and discard the flow-through. Discard the flow-through with collection tube and place the spin column into the fresh 2ml collection tube.

14. Add 500µl of Wash buffer-2 [Ethanol added] to the Purefast® spin column. Centrifuge at 10000rpm for 1min and discard the flow-through. Discard the flow-through with collection tube and place the spin column into the fresh 2ml collection tube.

15. Repeat Wash buffer-2 wash once.

16. Discard the collection tube. Insert Purefast spin column into fresh 1.5ml micro centrifuge tube. Centrifuge at **12000rpm** for **2 min** [Empty spin]. This step is essential to avoid residual ethanol. Discard the 1.5ml micro centrifuge tube.

17. Transfer the Purefast® spin column into a fresh 1.5 ml micro-centrifuge tube.

18. Add 60µl of Elution Buffer to the centre of Purefast® spin column membrane. Incubate 2 minute at room temperature.

19. Centrifuge at 10000rpm for 1 min and discard the Purefast spin column. Centrifuge tube now contains the eluted nucleic acid. Either use the directly in PCR or store at -80°C for later analysis.

Recommendation for Real-time PCR:
Use 5 - 20µl of elute

Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI Purefast Blood RNA mini spin prep kit is tested against predetermined specifications to ensure consistent product quality.

Explanations of symbols

In vitro diagnostic medical device

Catalogue number

Pack size – number of tests


Manufacturer

Manufactured by

HELINI *Biomolecules,*
Ohmlina, 26, 2nd Avenue,
Khuthubi Complex, Vettuvankeni,
Chennai 600115, Tamil nadu, INDIA
www.helini.in
info@helini.in
helinibiomolecules@gmail.com
+91-44-24490433 / +91-9382810333

www.helini.in