HELINI
Purefast
Viral RNA
Mini spin prep kit

Instructions for use

For use with: Human Plasma & Serum

CE
IVD

REF 2002

Σ 25/50/100/250 Prep

HELINI Biomolecules, Chennai, INDIA

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Intended Use

The HELINI Purefast viral RNA mini spin prep kit is a spin column based rapid and cost-effective small-scale preparation of high-quality Viral RNA from human plasma and serum. Purified viral RNA can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests	250 tests
Carrier RNA	5µl	125µl	250µl	0.5ml	1.25ml
Lysis buffer	560µl	15ml	30ml	60ml	150ml
Elution Buffer	60µl	2ml	4ml	8ml	15ml
Wash Buffer-1*	600µl	13ml	26ml	52ml	130ml
Wash Buffer-2*	600µl	6ml	12ml	24ml	48ml
Spin columns with collection tube	1	25	50	100	250
Collection tubes	3	75	150	300	750

***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.
- Upon arrival, Carrier RNA should be stored in -20°C.
- Remaining 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:2002– 25 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	13ml	6ml
Ethanol [96 – 100%] to add	9ml	16ml
Total volume	**22ml**	**22ml**

	Cat.No:2002 – 50 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	26ml	12ml
Ethanol [96 – 100%] to add	18ml	32ml
Total volume	**44ml**	**44ml**

	Cat.No:2002 – 100 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	52ml	24ml
Ethanol [96 – 100%] to add	36ml	64ml
Total volume	**88ml**	**88ml**

	Cat.No:2002 – 250 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	130ml	60ml
Ethanol [96 – 100%] to add	90ml	160ml
Total volume	**220ml**	**130ml**

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 >13000 x g (12000-14000 rpm, depending on the rotor type).

Adjustment of sample volume:

If your sample volume is less than 140µl, the sample volume should be adjusted with PBS/TE buffer.

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

Procedure:

1. Transfer 560µl of Lysis buffer into sterile 1.5ml centrifuge tube.

2. Pipette mix 5µl of carrier RNA into lysis buffer.

3. Add 140µl of Plasma/Serum. Mix well by pulse vortex for 15 seconds. [Option: If you are using Internal control template to monitor extraction efficiency, please **add 5µl of Internal control template**]

4. Centrifuge few seconds to bring down drops to the bottom of the tube.

5. Incubate in room temperature for 10min.

6. Add 560µl of 100% Ethanol and mix well by vortex for 20seconds. Spin down few seconds to bring down drops to bottom of the tube.

7. Transfer 630µl of sample-lysis mix into the Purefast® spin column. Centrifuge at 8000rpm for 1 min. Discard the flow-through and place the spin column back into the same collection tube.

8. Transfer remaining entire [630µl] sample-lysis mix into the Purefast® spin column. Centrifuge at 8000rpm for 1 min. Discard the collection tube containing filtrate. Place spin column into fresh collection tube.

9. Add 600µl of Wash buffer-1 [Ethanol added] to the Purefast® spin column. Centrifuge at 8000rpm for 1min Discard the collection tube containing filtrate. Place spin column into fresh collection tube.

10. Add 600µl of Wash buffer-2 [Ethanol added] to the Purefast® spin column. Centrifuge at 10000rpm for 1min. Discard the collection tube containing filtrate. Place spin column into fresh collection tube.

11. Centrifuge at **13000rpm** for **2 min** [Empty spin]. This step is essential to avoid residual ethanol. Discard the collection tube with flow-through.

12. Transfer the Purefast® spin column into a fresh 1.5 ml micro-centrifuge tube [Not provided with the kit].

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

14. Centrifuge at 8000rpm for 1 min and discard the Purefast spin column. Micro 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 viral 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

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