HELINI
Purefast
Viral nucleic acid
Mini spin prep kit

Instructions for use

For use with: Plasma, Serum, Swab cell pellet, CSF, other body fluids, VTM and fresh whole human blood

2001

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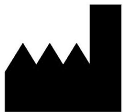

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Intended Use

The HELINI Purefast viral nucleic acid mini spin prep kit is a spin column based rapid and cost-effective small-scale preparation of high quality viral nucleic acid from human blood, plasma, serum swab cell pellet, CSF, other body fluids and VTM. Purified viral nucleic acid can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests
Carrier RNA	10µl	125µl	250µl	0.5ml
Proteinase K	20µl	0.5ml	1ml	2 x 1ml
Lysis buffer	250µl	6.5ml	13ml	26ml
Elution Buffer	60µl	2.5ml	5ml	10ml
Wash Buffer-1*	600µl	13ml	26ml	52ml
Wash Buffer-2*	600µl	6ml	12ml	24ml
Spin columns with collection tube	1	25	50	100
Collection tubes	3	75	150	300

***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, Proteinase K and 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
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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:2001– 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:2001 – 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:2001 – 100 prep	
	Wash buffer-1	**Wash Buffer-2**
Concentrated Buffer	52ml	24ml
Ethanol [96 – 100%] to add	36ml	64ml
Total volume	**88ml**	**88ml**

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.

Sample requirement/preparation:

Plasma/Serum/CSF/body liquids/fresh whole human blood:
Gently mix well and transfer 0.15ml for the purification.

Viral carrier media containing Swab [VTM]:
Vortex well with swab inside & safely discard the swab. Again, vortex well and transfer 0.15ml for the purification

Cervical Swabs stored in cytology media:

- Vortex well the tube with swab and carefully discard the swab.
- Centrifuge the tube at 8000rpm for 5min. using micropipette, carefully discard the supernatant without disturbing the pellet.
- Add 2ml of sterile distilled water to the pellet and vortex well.
- Centrifuge at 10000rpm for 3min and discard the supernatant.
- Add 2ml of sterile distilled water to the pellet and vortex well.
- Centrifuge at 10000rpm for 2min and discard the supernatant.
- Vortex well the pellet to dislodge, add 200µl of sterile distilled water and use 150µl for purification.

Note: Set water bath or Dry bath to 56°C**.**

Procedure:

1. Add 20µl of Proteinase K to the bottom of a fresh 1.5ml centrifuge tube.

2. Add 150µl of sample [refer page.11].

3. Add 250µl of Lysis Buffer. Mix well by pulse vortexing for 10 seconds.

4. Add 5µl of Carrier RNA. [Optional: If you are using Internal control template to monitor extraction efficiency, please **add 5µl of Internal control template**]

5. Mix immediately by brief vortexing and centrifuge few seconds to bring down drops to the bottom of the tube.

6. Incubate at 56ºC for 10min.

7. Add 250µl of [100%] ethanol and mix well by vortex for 10seconds. Spin down few seconds to bring down drops to bottom of the tube.

8. Transfer entire sample 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 **12000rpm** for **2 min** [Empty spin]. This step is essential to avoid residual ethanol. Discard the collection tube containing filtrate.

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

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

14. 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 Viral nucleic acid 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,*
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