HELINI
Purefast
Bacterial DNA
Mini spin prep kit

Instructions for use

For use with: Sputum, CSF, Urine pellet, Whole human blood, Pus and tissue.

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REF 2004

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HELINI Biomolecules, Chennai, INDIA

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Intended Use

The HELINI Purefast Bacterial DNA mini spin prep kit is a spin column based rapid and cost-effective small-scale preparation of high-quality nucleic acid from sputum, urine pellet, CSF, whole human blood, Pus, tissue and body fluids. Purified nucleic acid can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests
Lysozyme	10µl	0.5ml	1ml	2 x 1ml
Proteinase K	20µl	0.5ml	1ml	2 x 1ml
Tissue lysis buffer	200µl	5ml	10ml	20ml
Digestion buffer	180µl	5ml	10ml	20ml
Binding buffer	200µl	5ml	10ml	20ml
Elution Buffer	60µl	2.5ml	5ml	10ml
Wash Buffer-1*	500µl	9ml	18ml	36ml
Wash Buffer-2*	2x500µl	6ml	12ml	24ml
Spin columns with collection tube	1	25	50	100
Micro pestle	1	5	10	10

***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 Lysozyme 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;

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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:2004– 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:2004 – 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:2004 – 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.

Sample requirement/preparation:

Sputum sample:

1. To the container having sputum, Add equal volume of 4M NaoH-NALC solution [freshly prepared] and mix well. Incubate for 45min to 1hour. Intermediate mixing is required for effective digestion.

2. Add equal volume of sterile distilled water and Mix well using sterile glass rod. Transfer into 15ml or 50ml centrifuge tube.

3. Centrifuge at 8000rpm for 10min. Discard the supernatant. Vortex well to dislodge the pellet.

4. Dissolve the pellet in 1 or 2ml of TE Buffer or PBS or sterile distilled water. [Volume can be adjusted based on the cell pellet]. Transfer 0.5ml [depends on the cell pellet turbidity] into fresh 1.5ml centrifuge tube.

5. Centrifuge at 8000rpm for 5min. Discard the supernatant. Vortex well to dislodge the pellet.

6. DNA purification – Proceed to Step-2 main protocol page-16.

Procedure for DNA purification from Tissue samples:

1. Transfer 5 -10mg of tissue in a fresh 1.5ml centrifuge tube.

2. Add 200µl of tissue lysis buffer, using micro pestle, homogenise well.

3. Add 20µl of Proteinase K and mix well by pulse vortex.

4. Incubate at 56ºC for 45minutes.

5. Centrifuge at 6000rpm for 5min. Transfer the supernatant into fresh 1.5ml centrifuge tube.

6. Add 200µl Binding Buffer and Mix well by pulse vortex.

7. Proceed with Step 7 of DNA Purification protocol. [PAGE-16]

Whole Blood / liquid / CSF sample processing:

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

2. Add 200µl of whole human blood or CSF or body fluids.

3. Add 200µl of Binding Buffer. Mix well by pulse vortex for 15 seconds. Centrifuge few seconds to bring down drops to bottom of the tube. [Option: If you are using Internal control template to monitor extraction efficiency, please **add 5µl of Internal control template**]

4. Incubate at 56ºC for 15min.

5. Proceed from step 7 in DNA purification protocol. [PAGE-16]

Pus Sample

1. Transfer a loop of [10µl - 20µl] of sample into 200µl of PBS or TE buffer or Sterile distilled water. Vortex well.

2. Add 200µl of Binding Buffer and 20µl of Proteinase K. Mix well by pulse vortex for 15 seconds. Centrifuge few seconds to bring down drops to bottom of the tube. [Option: If you are using Internal control template to monitor extraction efficiency, please **add 5µl of Internal control template**]

3. Incubate at 56ºC for 15min and Proceed from step 7 in DNA purification protocol. [PAGE-16]

Urine Sample:

Note: As soon as the sample collected, add 0.5ml of EDTA to 4.5ml of Urine sample.

1. Transfer 5ml of urine sample into 15ml centrifuge tube and add equal volume of sterile PBS or distilled water. Mix well by inverting several times.

2. Centrifuge at 8000rpm for 5min. Discard the supernatant.

3. Suspend pellet in 2ml of PBS/TE Buffer/Sterile distilled water. [Volume of TE or PBS can be adjusted based on the cell pellet] centrifuge at 8000rpm for 5min.

4. Discard the supernatant. Vortex the pellet to dislodge.

5. Proceed with Step 2 of DNA purification protocol [Page-16]

Note: Set water bath or Dry bath to 56°C**.** Warm elution buffer in water bath.

Procedure:

1. Suspend bacterial pellet by vortex.

2. Add 180µl of Digestion buffer and 20µl of Lysozyme. Gently vortex for 10 seconds and briefly centrifuge to remove drops from inside the lid of the tube.

3. Incubate at 37ºC for 15min.

4. Add 200µl of Binding Buffer and 20µl of Proteinase K, Mix well by pulse vortex. [If you are using Internal control template, add 5µl of Internal control template and mix well].

5. Briefly centrifuge to remove drops from inside the lid of the tube.

6. Incubate at 56ºC for 15min.

7. Add 200µl absolute ethanol [100%] and mix well by inverting several times.

8. Pipette entire sample into the Purefast® spin column. Centrifuge at 8000rpm for 1 min. Discard the flow-through and place the column back into the same collection tube.

9. Add 500µl of Wash buffer-1 [Ethanol added] to the Purefast® spin column. Centrifuge at 10000rpm for 1min and discard the flow-through. Place the column back into the same collection tube.

10. Add 500µl of Wash buffer-2 [Ethanol added] to the Purefast® spin column. Centrifuge at 10000rpm for 1min and discard the flow-through. Place the column back into the same collection tube.

11. Repeat Wash buffer-2 wash once.

12. 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.

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

14. Add 100µl of the pre-warmed Elution Buffer to the centre of Purefast® spin column membrane. Take care not to contact the membrane with the pipette tip.

15. Incubate for 2 min at room temperature and Centrifuge at 13000rpm for 1 min. Discard the spin column and store the purified DNA at -20°C. Note: Elution volume can be adjusted from 30µl to 100µl.

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 Bacterial DNA 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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