HELINI
Purefast
Tissue DNA
Mini spin prep kit

Instructions for use

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IVD

REF 2007

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HELINI Biomolecules, Chennai, INDIA

www.helini.in

Intended Use

The HELINI Purefast tissue DNA mini spin prep kit is a spin column based rapid and cost-effective small-scale preparation of high-quality genomic DNA from fresh and frozen tissue samples. Purified DNA can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests
Proteinase K	20µl	0.5ml	1ml	2 x 1ml
Tissue lysis buffer	0.5ml	12.5ml	25ml	50ml
Binding buffer	0.2ml	5ml	10ml	20ml
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	3	75	150	300
Shredder spin column	1	25	50	100

***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 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:2007– 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:2007 – 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:2007 – 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 sample volume to be used more, Scale up buffers volume accordingly.

Note: Before starting the purification reaction, set water bath to 56ºC and warm up the Elution buffer to 56ºC.

Procedure:

1. Transfer 5 -20mg of tissue in a fresh 1.5ml centrifuge tube. [It is recommended to cut the tissue as much as smaller using sterile scalpel and then transfer to 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. Briefly centrifuge to remove drops from inside the lid of the tube.

4. Incubate at 56ºC for 1 to 3 hours (OR overnight). [Recommended to use water bath]

5. Insert fresh Shredder spin column in to fresh 2ml centrifuge tube, Transfer the whole lysate to Shredder spin column. Centrifuge at 8000rpm for 1min.

6. Discard the Shredder spin column, add 200µl Binding Buffer to the flow through lysate and Mix well by pulse vortex. [If you are using internal control template, add 5µl of internal control template and mix well].

7. Add 220µl absolute ethanol [100%] and mix well by inverting several times. Briefly centrifuge to remove drops from inside the lid of the tube.

8. Transfer entire sample 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.

9. 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.

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

11. Repeat Wash buffer-2 wash once.

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

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

14. Add 100 to 200µl of Elution Buffer to the centre of Purefast® spin column membrane. Incubate 2 minute at room temperature.

15. 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 Tissue 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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