HELINI
Purefast
MicroRNA
Mini spin prep kit

Instructions for use

For use with: Plasma, serum and cell culture

CE IVD

REF 2008

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HELINI Biomolecules, Chennai, INDIA

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Intended Use

HELINI Purefast MicroRNA Mini spin prep Kit is designed for rapid and cost-effective small-scale preparation of high-quality microRNA from plasma, serum and cell pellets. Purified microRNA can be used directly in RT-PCR/PCR.

Kit components

Components	Volume Per reaction	25 tests	50 tests	100 tests
Lysis buffer	1ml	25ml	50ml	100ml
Elution Buffer	30µl	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

***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:2008– 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:2008 – 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:2008 – 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.

Procedure

1. Transfer 0.2ml of Plasma or serum or cell pellet suspended in 0.2ml sterile water or PBS into fresh 2ml centrifuge tube. [Note: use only 2ml micro centrifuge tube]

2. Add 1ml of Lysis buffer and mix well by brief vortex. *[If you are using internal control micro RNA to check the efficiency of the purification, add and mix well 10µl of HELINI internal control template at this step]*

3. Incubate at room temperature for 5 minutes.

4. Add 200µl of Chloroform and mix well by brief vortex.

5. Incubate at room temperature for 5 minutes.

6. Centrifuge at 12000rpm for 5min at 4ºC. After centrifugation, the sample separates into 3 phases: an upper, colourless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase.

7. Transfer the upper aqueous phase to a new 2ml collection tube. Avoid transfer of any interphase material. Add 1.5volumes of 100% ethanol and mix thoroughly by pipetting up and down several times. Do not centrifuge. Continue without delay with step 8.

8. Pipette 700µl including any precipitate that may have formed, to Purefast® spin column attached with 2 ml collection tube. Close the lid gently and centrifuge at 10000 rpm for 1 min at room temperature. Discard the flow-through. Place the spin column back into the collection tube.

9. Pipette the remaining samples to Purefast® spin column and centrifuge at 10000 rpm for 1 min at room temperature. Discard the flow-through. Place the spin column back into the collection tube.

10. Add 500µl Wash Buffer-1 to the Purefast® spin column. Close the lid gently and centrifuge for 1min at 10000 rpm. Discard the flow-through. Place the column back into the collection tube.

11. Add 500µl Wash Buffer-2 to the Purefast® spin column. Close the lid gently and centrifuge for 1min at 10000rpm. Discard the flow-through. Place the column back into the collection tube.

12. Repeat Wash buffer-2 wash once.

13. Discard the collection tube. Insert Purefast spin column into fresh 1.5ml micro centrifuge tube [not included]. Centrifuge at **12000rpm** for **2 min** [Empty spin]. This step is essential to avoid residual ethanol. Discard the 1.5ml micro centrifuge tube.

14. Transfer the Purefast® spin column into a fresh 1.5 ml micro-centrifuge tube (not included).

15. Add 30 to 50µl of Elution Buffer to the centre of Purefast® spin column membrane. Incubate 2 min at room temperature.

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

Recommendation for RT-PCR:

Use 10 - 20µl of elute for reverse transcription reaction.

Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI Purefast microRNA 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