Quick Protocol

DNJia CF Kit

Free circulating nucleic acid isolation based on silica technology

For free-circulating DNA, RNA, miRNA, and viral nucleic acids

from

Serum
Plasma
Urine
Cell-free body fluids

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Kit Content

Component	50 preps
CFL	2 x 60 ml
CFB (concentrate)	5 x 36 ml
ERW1 (concentrate)	19 ml
ERW2 (concentrate)	13 ml
ERR	10 ml
Carrier RNA	620 µg
CF-Protease	2*8.5 ml
CF- Column	50
Collector	50
Connector	50
Collection Tube	50

Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 4. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

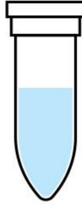
Table 1: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
ERW1	19 ml	25 ml	44 ml
ERW2	14 ml	32 ml	46 ml

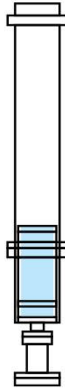
Procedure of silica-based Free circulating nucleic acid isolation in quick look

Quick Protocol

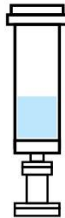
Step1: Lyse



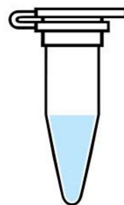
Step2: Bind



Step3: Wash Vacuum



Step4: Elute Vacuum



Pure nucleic acids

Protocol: Purification of Circulating Nucleic Acids from 1 ml, 2 ml, or 3 ml Samples

Sample Type: Serum, Plasma, Urine

Some tips to know

- Green denotes sample volumes of 1 ml serum, plasma or urine; blue denotes sample volumes of 2 ml serum, plasma or urine; red denotes sample volumes of 3 ml serum, plasma or urine.
- All centrifugation steps are carried out at room temperature (15–25°C).
- Switch off vacuum between steps to ensure that a consistent, even vacuum is applied during protocol steps.
- Equilibrate samples to room temperature.
- If samples are less than 1 ml, 2 ml, or 3 ml, bring the volumes up to 1 ml, 2 ml, or 3 ml with phosphate buffered saline.
- Set up your vacuum device.
- Adjust a heating block to 60°C for use with 50 ml centrifuge tubes in step 4.
- Adjust a heating block to 56°C for use with 2 ml collection tubes in step 14.
- Equilibrate ERR to room temperature for elution in step 15.
- Add carrier RNA reconstituted in ERR to CFL refer to buffer preparation part
- Add appropriate amount of 96-100 ethanol to ERW1 and ERW2, refer to buffer preparation part.
- Add appropriate amount of isopropanol to the CFB buffer according to their label, refer to buffer preparation part
- If CFL forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Pipet 100 µl, 200 µl, or 300 µl CF-Protease into a 50 ml microtube (not provided).
2. Add 1 ml, 2 ml, or 3 ml of serum, plasma or urine to the 50 ml tube.
3. Add 0.8 ml, 1.6 ml, or 2.4 ml prepared CFL (refer to CFL preparation part) then, pulse-vortexing for 30 s.
Make sure that a visible vortex forms in the tube. In order to ensure efficient lysis, it is essential that the sample and CFL are mixed thoroughly to yield a homogeneous solution.
Note: Proceed immediately to step 4 to start the lysis incubation.
4. Incubate at 60°C for 30 min.
5. Place the tube back on the lab bench and unscrew the cap.
6. Add 1.8 ml, 3.6 ml, or 5.4 ml CFB to the lysate in the tube. Then, pulse vortexing for 15–30 s.
7. Incubate the lysate–CFB mixture for 5 min on ice.
8. Insert the CF-Column into the Connector on the vacuum device. Insert a 20 ml tube extender into the open CF-Column or continuously add the lysate to the CF-Column until it is passed completely.
Make sure that the tube extender is firmly inserted into the CF-Column in order to avoid leakage of the sample.
Note: Keep the collection tube for the dry spin in step 13.
9. Carefully transfer the lysate–CFB mixture from step 7 into the tube extender of the CF-Column. Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the tube extender.
Please note that large sample lysate volumes (about 11 ml when starting with 3 ml sample) may need up to 10 minutes to pass through the membrane by vacuum force. For fast and convenient release of the vacuum pressure, the vacuum regulator should be used (part of the vac connecting system).
Note: To avoid cross-contamination, be careful not to move the tube extenders over neighboring CF-Columns.
10. Add 600 µl ERW1 to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of ERW1 has been drawn through

the CF-Column, switch off the vacuum pump, and release the pressure to 0 mbar.

11. Add 750 µl ERW2 to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of ERW2 has been drawn through the CF-Column, switch off the vacuum pump and release the pressure to 0 mbar.

12. Add 750 µl of ethanol (96–100%) to the CF-Column. Leave the lid of the column open, and switch on the vacuum pump. After all of the ethanol has been drawn through the CF-Column, switch off the vacuum pump and release the pressure to 0 mbar.

13. Close the lid of the CF-Column. Remove it from the vacuum manifold, and discard the Connector. Place the CF-Column in a clean 2 ml collection tube, and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

14. Place the CF-Column into a new 2 ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

15. Discard the 2 ml collection tube from step 14. Place the CF-Column in a clean 1.5 ml microtube. Carefully add 20–150 µl of ERR into the center of CF-Column. Incubate at room temperature for 3 min.

Important: Equilibrate ERR to room temperature (15–25°C). Elution volume is flexible and can be adapted according to the requirements of downstream applications.

16. Centrifuge the microtube at full speed (20,000 x g; 14,000 rpm) for 1 min.