CedExtra Purification Kit (100 prep)

Product Name

CedExtra Purification Kit

Packaging Specifications

100 Tests per box

Product Code

110120

Intended Use

Extraction of high-quality DNA/RNA from human, viral, bacterial, and fungal samples, including blood, plasma, serum, buffy coat, body fluid, stool, cell culture, tissue, and FFPE samples, and Cytology specimens.

Test Principal

This kit is based on silica column extraction technology. The lysis buffer contains detergents, guanidinium isothiocyanate, and Tris buffer. The wash buffers contain guanidinium hydrochloride. The extracted DNA can be used for a wide range of downstream applications, including reverse hybridization and PCR-based methods.

Kit Components

Components	Amount
Lysis Buffer (LB)	22 ml
Tissue Lysis Buffer (TL)	22 ml
Binding Buffer (BB)	50 ml
Wash Buffer 1 (WB1)	40 ml
Wash Buffer 2 (WB2)	2x12.5 ml
Elution Buffer (EB)	30 ml
Columns	100 pcs
Collection Tubes	200 pcs
Proteinase K	2x 1.2 ml

Storage Conditions and Shelf Life

Store the kit at room temperature (15–30 °C), and away from sunlight. Keep Proteinase K at -20 °C. Stable for 24 months from manufacturing date if stored properly.

Required Equipment and Materials

- Microcentrifuge (14,000 × g)
- Biosafety cabinet
- Vortex mixer
- Nuclease-free filtered tips
- Adjustable volume pipette
- RNase-free sterile microtubes
- Biohazard waste container
- Absolute ethanol
- Disposable gloves

Precautions and Warnings

• Please read this user manual carefully and strictly follow the instructions in the user manual.
• This protocol must be performed only by trained personnel familiar with molecular diagnostic techniques.
• All specimens should be considered potentially infectious and handled accordingly under a laminar flow hood.
• Pay attention to the kit expiration date.
• Dispose of waste directly into disinfectant solutions.
• Lysis buffer (LB), tissue lysis buffer (TL), and wash buffer 1 (WB1) contain Guanidinium Isothiocyanate/SDS: harmful if swallowed or inhaled, may cause irritation to eyes, skin, and respiratory system.
• In case of contact, rinse immediately and seek medical advice if irritation persists.
• Wear protective gloves, a lab coat, and eye/face protection during handling.

Sample Collection, Handling, and Storage

- Collect samples in sterile containers.
- Transport samples to the laboratory under biosafety conditions.
- Extract nucleic acids immediately or store samples at -20°C, 4°C, or room temperature depending on sample type and stability.

Solution Preparation Before Starting the Protocol

1- Add 27 mL of Ethanol to WB1
2- Add 65 mL of Ethanol to WB2

***Absolute Ethanol or 96% Ethanol should be used.

Sample Preparation and Nucleic Acid Extraction Procedure

Sample Type

Blood, body fluid including CSF, synovial fluid, pleural fluid, and urine, semen, cytology samples, and stool.

Sample Preparation

1. Blood

For whole blood, mix the vial thoroughly using a hematology or CBC mixer. Invert the vial multiple times to ensure proper mixing.

For dried blood spots on Guthrie paper:

- Place the sample paper into a 1.5 mL tube containing 250 µL of physiological saline solution (NaCl 0.9%) and incubate at room temperature for 15 minutes.
- Vortex for 10 seconds, remove the paper, and use the solution in the tube for extraction.

2. Body Fluid (low cell count):

- Take 1.5 mL of sample and transfer it into a 2 mL microtube. Centrifuge at 13,000 rpm for 5 minutes.
- Discard the supernatant and add 200 µL of distilled water to the pellet for extraction.

3. Liquid-Based Cytology (Cervical Samples, LBC, Self-Sampling):

- Vortex the sample-containing tube for 10 seconds to detach cells from the brush or pad. Transfer 400 µL of the sample into a 1.5 mL microtube. Add 600 µL of PBS, Tris buffer, or physiological saline solution and centrifuge at maximum speed for 30 seconds. Discard the supernatant.
- Add 600 µL of buffer, vortex for 5 seconds, centrifuge again at maximum speed for 30 seconds, and discard the supernatant. Add 200 µL of buffer to the pellet for extraction.

4. Stool

- Take approximately 1 mm^3 of stool and add 1 mL of 96% ethanol. Vortex for 5 seconds and incubate at room temperature for 1 minute. Centrifuge at maximum speed for 30 seconds and discard the supernatant. Repeat this step.
- Add 1mL of PBS, Tris buffer, or physiological saline solution to the tube, vortex for 5 seconds, and incubate at room temperature for 1 minute. Centrifuge at maximum speed (>13,000 rpm) for 30 seconds and discard the supernatant. Repeat this step. Add 200 µL of buffer to the pellet for extraction.

Nucleic Acid Extraction Procedure

1. Add 200 µL of Lysis Buffer (LB) to a 1.5 mL tube containing 200 µL of the prepared sample (according to the sample preparation protocol). Then, add 20 µL of Proteinase K, vortex for 5 seconds, and spin briefly.

2. Incubate the mixture on a heat block or thermomixer (400 rpm) at 56°C for 20 minutes. Then, vortex for 5 seconds, and spin briefly.

3. Add 400 µL of Binding Buffer (BB) to the lysate and sample mixture, vortex for 5 seconds, and spin briefly.

4. Transfer 800 µL of the lysate mixture into a spin column placed in a collection tube and centrifuge at 10,000 × g for 1 minute.

"If the lysate does not fully pass through the column due to the age of the blood sample, centrifuge again at 13,000 rpm for 1 minute."

5. Place the column into a new collection tube, add 600 µL of Wash Buffer 1 (WB1), and centrifuge at 10,000 × g for 1 minute.

* At this stage, the paper layer should be free of hemoglobin residues. If brown coloration remains for any reason, repeat this step.

6. Place the column into a new collection tube, add 600 µL of Wash Buffer 2 (WB2), and centrifuge at 10,000 × g for 1 minute.

7. Place the column into a new collection tube, add 650 µL of Wash Buffer 2 (WB2), and centrifuge at 10,000 × g for 1 minute.

8. Place the column into a new collection tube and centrifuge at maximum speed (>13,000 rpm) for 1 minute.

9. Place the column into a 1.5 mL microtube, add 100 µL of Elution Buffer (EB), and incubate at room temperature for 1 minute. Then centrifuge at maximum speed for 1 minute to collect the eluted nucleic acid.

10. The eluted solution is ready for all molecular tests and should be stored at -20°C for future use.

Sample Type

Cell culture, Tissue, and FFPE.

Sample Preparation

1. Fixed Tissue Samples

Cut a piece approximately 1 mm in size and mince it on a glass slide using a scalpel. Transfer the minced tissue into a 1.5 mL microtube.

2. Paraffin-Embedded Tissue Blocks (FFPE)

- Cut a 5 µm section and place it into a 1.5 mL microtube. Add 1 mL of xylene, vortex for 5 seconds, and incubate at room temperature for 1 minute. Centrifuge at maximum speed for 30 seconds, discard the supernatant, and repeat this xylene wash once more.
- Add 1 mL of absolute ethanol to the microtube containing the tissue, vortex for 5 seconds, and incubate at room temperature for 1 minute. Centrifuge at maximum speed (>13,000 rpm) for 30 seconds, discard the supernatant, and repeat the ethanol wash one more time.
- After discarding the final supernatant, place the microtube on a heat block at 56°C to evaporate any remaining ethanol.

Nucleic Acid Extraction Procedure

1. Add 200 µL of Tissue Lysis Buffer (TL) to a 1.5 mL tube containing the prepared tissue sample (according to the sample preparation protocol). Then add 20 µL of Proteinase K, vortex for 5 seconds and spin briefly,

2. Incubate the mixture on a thermomixer at 56°C for 1 to 24 hours at 400 rpm. If using a heat block, vortex and spin the tube for 5 seconds every 20 minutes to ensure complete digestion of the tissue.

3. Add 200 µL of Lysis Buffer (LB) to the mixture of tissue and TL lysis, vortex for 5 seconds, and spin briefly.

4. Incubate on a thermomixer (400 rpm) or on a heat block at 56°C for 10 minutes, then vortex and spin briefly for 5 seconds.

5. Add 400 µL of Binding Buffer (BB) to the lysed tissue mixture, vortex for 5 seconds, and spin briefly.

6. Transfer 800 µL of the lysate mixture into a spin column placed in a collection tube and centrifuge at 10,000 × g for 1 minute.

7. Place the column into a new collection tube, add 600 µL of Wash Buffer 1 (WB1), and centrifuge at 10,000 × g for 1 minute.

8. Place the column into a new collection tube, add 600 µL of Wash Buffer 2 (WB2), and centrifuge at 10,000 × g for 1 minute.

9. Place the column into a new collection tube, add 650 µL of Wash Buffer 2 (WB2), and centrifuge at 10,000 × g for 1 minute.

10. Place the column into a new collection tube and centrifuge at maximum speed (>13,000 rpm) for 1 minute.

11. Place the column into a 1.5 mL microtube, add 100 µL of Elution Buffer (EB), and incubate at room temperature for 1 minute. Then centrifuge at maximum speed for 1 minute to collect the eluted nucleic acid.

12. The eluted solution is ready for all molecular tests and should be stored at -20°C for future use.

***Optional modifications for improved yield, concentration, and convenience (beyond the standard method, which provides ~70% -90% recovery):

- **High Yield Elution:** Perform elution in two separate steps using the buffer volume specified in the protocol. This allows recovery of approximately 90–100% of the nucleic acid bound to the resin.
- **High Concentration Elution:** Perform elution in a single step using 40 µL of elution buffer. The concentration of DNA will be about 30% higher compared to the standard elution.
- **High Yield and High Concentration Elution:** Add 50 µL of elution buffer (half of the total volume specified in the protocol), incubate for 3 minutes, and centrifuge. Then add the remaining 50 µL, incubate, and centrifuge again. Under these conditions, approximately 85–100% of the nucleic acid bound to the column can be recovered at a high concentration in the same elution volume as the standard protocol.

Tip: For all of the above approaches, elution efficiency can be further improved by using an elution buffer pre-warmed to 70°C.

ndations:

- For *Hepatitis Virus* nucleic acid extraction, it is recommended to increase the incubation time in the lysis step to 30 minutes.
- For optimal extraction of *Mycobacteria* and Gram-positive bacteria, add 20 µL of Lysozyme (10 mg/mL) to the sample (depending on the type of initial sample preparation) and incubate at room temperature for 30 minutes before starting the extraction process. Extraction of these samples should then be carried out according to the tissue sample protocol.
- Extraction of fungal nucleic acids should also be performed according to the tissue sample protocol.

Troubleshooting

Issue	Possible Causes	Recommendations
Low yield and purity of nucleic acid	Kit stored under inappropriate conditions	Store the kit at 15–25 °C immediately after delivery.
	Buffers or other reagents stored improperly	Store all buffers at 15–25 °C. After each use, close reagent bottles tightly to prevent pH instability or contamination due to improper sealing. Store all reagents at the temperatures recommended in the instructions.
	Ethanol was not added to Wash Buffer 1 and 2	Add absolute ethanol to the wash buffers before use. Mix thoroughly after adding ethanol and store at 15–25 °C. Clearly label wash buffer vials to indicate whether ethanol has been added.
	Reagents and samples were not mixed completely	Mix the tube contents thoroughly after adding each reagent.
	Frozen blood sample not mixed properly after thawing	Thaw frozen blood at room temperature and gently mix by inverting the tube.

Issue	Possible Causes	Recommendations
	Incomplete lysis	Add an additional 20 µL of Proteinase K, vortex, and allow sufficient incubation time (up to 4 hours at 56 °C) until lysis is complete.
	The Binding Buffer was not added to the lysate	Ensure that the Binding Buffer is completely mixed with the lysate before applying to the silica column.
	Inadequate washing	After the washing step with Wash Buffer 2, wash the column with 600 µL of absolute or 96% ethanol.
Gel-like substances in the elution	Use of aged blood samples	Remove gel-like substances by centrifugation. It is recommended to use fresh blood. If needed, 8 M NaOH can be used as a wash buffer.
Low nucleic acid recovery due to Elution buffer	The Elution Buffer used is not optimized for alkaline pH	Use the recommended Elution Buffer with optimal alkaline pH. Do not use distilled water for washing nucleic acids from the column.
	Incomplete washing of the column by the elution buffer	Repeat the drying step (Step 7) to ensure complete removal of wash buffers. Do not use cold Elution Buffer. Incubate on the column for at least 1 minute; incubation up to 5 minutes may improve recovery. Preferably use pre-warmed Elution Buffer at 70°C.
Poor performance of the extraction column	Improper storage; the column may have been discharged.	Add 100 µL of 3 M NaOH to the extraction column and centrifuge at 10,000 × g for 1 minute. Add 100 µL of distilled water to the column and centrifuge at 10,000 × g for 1 minute. After this treatment, the column remains stable for up to 6 hours. After this period, it should not be used.
	Incomplete digestion by Proteinase K	Cut the tissue into small pieces. Prepare the tissue according to the standard tissue preparation protocol. Increase Proteinase K incubation using one of the following methods: 1. Incubate the tissue sample overnight with Proteinase K. During this period, vortex the sample at least three times. 2. Incubate the tissue sample for 3–4 hours with 20 µL of Proteinase K, then add an additional 30 µL of Proteinase K and incubate for another 1–2 hours.

References

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2. AL-SHUHAIB, S. A., & BAQUR, M. (2017). A universal, rapid, and inexpensive method for genomic DNA isolation from the whole blood of mammals and birds. Journal of Genetics, 96(1), 171-176.
3. Koshy, L., Anju, A. L., Harikrishnan, S., Kutty, V. R., Jissa, V. T., Kurikesu,. (2017). Evaluating genomic DNA extraction methods from human whole blood using endpoint and real-time PCR assays. Molecular biology reports, 44(1), 97-108.
4. Chacon Cortes, D. F., & Griffiths, L. (2014). Methods for extracting genomic DNA from whole blood samples: current perspectives. Journal of Biorepository Science for Applied Medicine, 2014(2), 1-9.

Explanation of used symbols

	Please read the Instruction for Use
	Warning, please read Instructions for Use
RUO	For Research Use Only
REF	Catalog number
LOT	Lot number
	Temperature Limit
	Expiry date
	Manufacturer

Manufacturer information

Arraytech Diagnostics SL
Calle Vía de los Poblados 1, Edificio A, Planta 1, Oficina C 28033 Madrid Spain
Tel.: +34 918603594 |E-mail: info@arraytech.es |Web: www.arraytech.es