GeneDia

Column-based Cell-Free DNA isolation Kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK0750R

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Introduction

The **GENEDIA™ Column base Cell-Free DNA isolation Kit** provides a rapid method for the isolation and purification of genomic DNA from Cell-Free samples. The **GENEDIA™ Column base Cell-Free DNA isolation Kit is** developed for scalable, rapid purification of high-quality DNA from serum samples. DNA purified with this kit can be used in a broad range of molecular biology downstream applications, such as sequencing, genotyping, and qPCR.

Kit Specifications

Purification is based on spin column chromatography using GENEDIA's proprietary resin as the separation matrix. The Cell-Free DNA is preferentially purified from other cellular components without the use of phenol or chloroform. First, the serum or plasma or other type of samples are lysed in the presence of chemotropic salts. Next, Absolute Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. GENEDIA's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the Cell-Free DNA will bind to the column while the contaminants will be removed in the flow through or retained on the top of the resin. The bound Cell-Free DNA is then washed with the provided Wash Solutions in order to remove any impurities and the purified Cell-Free DNA is eluted with CFEB.

Kit Components

Components	Product # EK0750R (50 preps)
CFLB	20 ml
CFWB1	12 ml
CFWB2	7 ml
CFEB	2.5 ml
CF carrier	0.5 ml
Spin Columns	50
Elution tubes	50
Product Insert	1

Storage Conditions

All components of the **GENEDIA™ Column base Cell-Free DNA isolation Kit should** be stored at 25 °C and are stable for 1 year.

Recommended Equipment and Reagents

- 58-80 °C incubator
- Sampler in 100 to 1000 Microliter size
- Sampler tips
- 1.5 mL micro centrifuge tubes
- Vortex
- Proteinase K (NOT provided)

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Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.

Notes Prior to Use

• Prepare a working concentration of the **CFWB1** by adding:

❖ **5 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **CFWB1**. This will give a final volume of 17 ml for **Product # EK0750R.**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **CFWB2** by adding:

❖ **10 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **CFWB2.** This will give a final volume of 17 ml for **Product # EK0750R.**

Sampling and Extraction Procedure

A. Sample preparation

The **GENEDIA™ Column base Cell-Free DNA isolation Kit** is compatible with serum samples separated from peripheral blood.

Note: *Sampling should be done in special tubes for cell free DNA (NOT provided). The unique preservative limits the release of genomic DNA, allowing isolation of high-quality cell-free DNA. Cell-Free DNA BCT has also been demonstrated to minimize the degradation of circulating tumor cells (CTCs). By limiting cell lysis, the specialized chemistry provides sample integrity during storage, shipping and handling of blood samples.*

B. Extraction procedure

1. Centrifuge the **serum sample** for **15 minutes** at **13000 rpm**. Transfer **1ml** of **supernatant** to a 2ml Micro tube.
2. Add **400 µL** of **CFLB (shake vigorously before use)** and **30 µL** of **Proteinase K (NOT provided) and 10 µL** of **CF Carrier** and close the cap. Incubate the mixture for **30 minutes** at **60°C**.
3. Cool the microtube containing sample to room temperature.
4. Add **700 µL** of **cold Ethanol** (NOT provided). Vortex for 30 seconds.
5. Incubate at **4°C** for **10 minutes**.
6. Assemble a Spin column with collection tube.
7. Transfer **750 µL** of lysate onto the column and incubate for **2 minutes** at **room temperature**.
8. Centrifuge at **8,000 x g** (~ 6,000 RPM) for **2 minutes**.
9. Discard the flow through. Reassemble the spin column with its collection tube.
10. Repeat 7-9 steps until all of the lysate has been drawn through the column.

Note: *Typically, samples will pass through the columns within ≤ 1 minute. If the entire volume has not passed, spin for an additional minute.*

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11. Add **300 µl** of **Buffer CFWB1** to the GENEDIA cf-DNA Spin column.
12. Centrifuge at **11,000 x g** (~ 10,000 RPM) for **1 minutes**.
13. Discard the flow through. Reassemble the spin column with its collection tube.
14. Add **300 µl** of Buffer **CFWB2** to the column.
15. Centrifuge for **1 minute at 11,000 x g** (~10,000 RPM).
16. Discard the flow through and reassemble the spin column with its collection tube.
17. Spin the column for **2 minutes** in order to thoroughly dry the resin.
18. Discard the collection tube.
19. Place the column into a fresh Elution tube provided with the kit.
20. Add **50 µl** of pre-heated **CFEB** to the column. Incubate the assembly at room temperature for 2 minute.
21. Centrifuge for **1 minute 12,000 x g** (~12,000 RPM).

Note: *If the entire volume has not been eluted, spin the column at 12,000 x g (~12,000 RPM) for 1 additional minute.*

Note: *For an improved yield, elute the sample twice and use after concentration process*

C. Storage of DNA

The purified DNA is ready for immediate use. For short term storage, keep the extracted cf-DNA in refrigerator at 2-8°C and for long term storage keep it in fridge at -20 to -70 °C.

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Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
No DNA purified	**Reagents added incorrectly**	Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence.
	Very low load of cf-DNA	Increase the volume of the starting sample (up to 5 ml).
	Incomplete elution	Larger elution volumes and longer incubation times can increase yield.
	Reagents added incorrectly	Make sure that buffers have been reconstituted correctly, and that reagents have been added in the correct order.
	Incomplete elution during preparation	Larger elution volumes and longer incubation times can sometimes increase yield. Multiple rounds of elution can also be performed.
Low DNA performance	**Ethanol has been carried over**	Centrifuge 2nd wash for 1 minute to ensure complete removal.
Small amounts or no nucleic acids in the eluate	**Nucleic acids degraded**	Samples should be processed immediately. If necessary, add DNase inhibitor to the sample. Create a nuclease-free environment and ensure that no nucleases are present. Use suitable tips and buffer reservoirs. Check that all buffers have been prepared and stored correctly.

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Isolation Method of Cell-Free DNA

Workflow

Centrifuge the serum sample for 15 min at 13000 rpm. 1ml of supernatant adding it to a 2ml Microtube.

Add 400 µL of CFLB & 30 µL of Proteinase K & 10 µL of CF Carrier and close the cap. Incubate the mixture for 30 min at 60°C.
Add 700 µL of cold Ethanol Vortex for 30 seconds & Incubate at 4°C for 10 min.

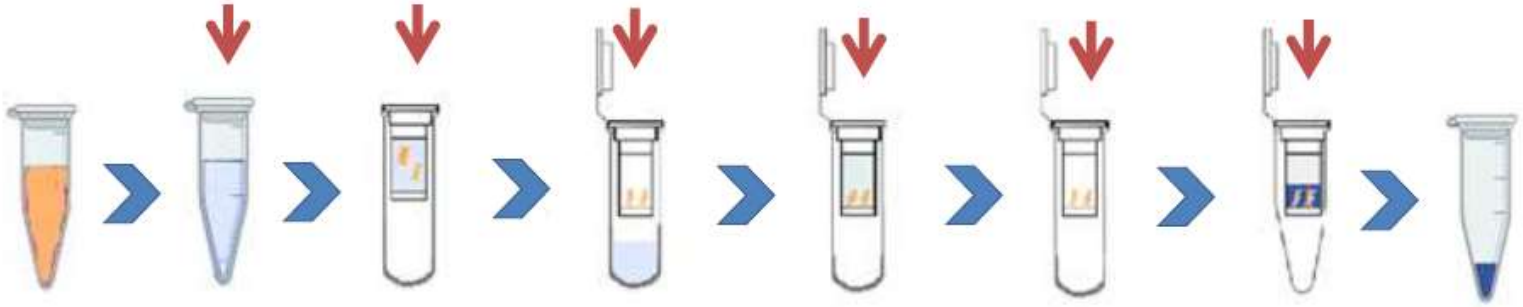
Transfer 750 µL of lysate onto the column and incubate for 2 min at RT.
Centrifuge at 8,000 x g for 2 min. Discard the flow through. Reassemble the spin column. Repeat these steps until all of the lysate has been drawn through the column

Add 300 µl CFWB1 and Centrifuge at 11,000xg 1 min Discard the flowthrough

Add 300 µl CFWB2 and Centrifuge at 11,000xg 1 min Discard the flowthrough

Place the column into an Elution tube Add 50 µl CFEB & Incubate for 2min at RT & Centrifuge 12,000xg 1 min

Purified Cell Free DNA



Sample Lysis | Nucleic acid Binding | Flow Through | 1st Wash | 2nd Wash | Elution

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