Plasma cfDNA Extraction Kit

REF 2-040

Σ 50 Extractions

18-25°C

1.	**Binding Buffer B**	4x 110 ml
2.	**Proteinase K**	2x 6.5 ml
3.	**Wash Buffer W1**	2x 18 ml
4.	**Wash Buffer W2**	38 ml
5.	**Elution Buffer E**	85 ml
6.	**Midi Columns**	5x 10
7.	**Micro Columns**	50
8.	**Collection Tubes**	50
9.	**Elution Tubes**	2x 25

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Instructions for use

I. INTENDED USE

Kit for the isolation of circulating cell-free DNA (cfDNA) from fresh or frozen human plasma prepared from blood collected on either PAXgene Blood ccfDNA Tubes (Qiagen), Cell-Free DNA BCT® Tubes (Streck), EDTA or Citrate. *For research use only.*

II. METHODOLOGY

The Plasma cfDNA Extraction Kit provides a reliable and simple procedure for isolating cfDNA from various amounts of plasma ranging from 1 ml to 4 ml. Purification is based on spin column chromatography that uses a proprietary resin separation matrix. The kit is designed to isolate all sizes of cfDNA from either fresh or frozen plasma samples. Moreover, this kit allows to elute the purified cfDNA into a flexible elution volume ranging from 25 µl to 50 µl. The purified plasma cfDNA is eluted in an Elution Buffer that is compatible with all downstream applications including PCR, qPCR, methylation-sensitive PCR and Southern Blot analysis, microarrays and next-generation sequencing (NGS).

Sample Type	Human blood plasma
Anti-coagulant or Plasma Preservative	EDTA or Citrate PAXgene Blood ccfDNA Tubes (Qiagen) Cell-Free DNA BCT® Tubes (Streck)
Sample Volume Range	1 - 4 ml
Minimum Elution Volume	25 µl
Maximum Elution Volume	50 µl
Size of purified DNA	≥ 50 bp

III. KIT COMPONENTS

See list of all kit components on page I.


Warning: Binding Buffer B contains guanidinium chloride
(H302, H315, H319; P280, P301+312+330, P302+352, P332/337+313)


Danger: Proteinase K
(H319, H334, H402; P280, P261, P264, P273)


Warning: Wash Buffer W1 contains guanidinium chloride
(H302, H315, H319; P280, P301+312+330, P302+352, P332/337+313)

Store all reagents at room temperature (18-25°C).
Optional: Proteinase K may be stored at 2-8°C for improved preservation.

IV. MATERIALS REQUIRED BUT NOT SUPPLIED

In addition to standard molecular biology laboratory equipment, the following is needed:

- Benchtop microcentrifuge (suitable for Micro Columns)
- Swinging bucket centrifuge (suitable for 15 ml tubes). *Do NOT use a fixed-angle rotor.*
- Thermoblock or waterbath capable of 60°C and holding 15 ml tubes
- 15 ml tubes (for centrifugation of Midi Columns)
- 96-100% ethanol

V. ASSAY PROCEDURE

1. Important Notes prior to use:

- Prepare a working solution of **Wash Buffer W1** by adding **24 ml** of **96-100% ethanol** to the supplied concentrate. This will give a final volume of 42 ml. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare a working solution of **Wash Buffer W2** by adding **90 ml** of **96-100% ethanol** to the supplied concentrate. This will give a final volume of 128 ml. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Preheat the thermoblock or waterbath to 60°C.
- Vortex Proteinase K each time before use.
- Ensure that all solutions are at room temperature prior to use, and that no precipitates have formed. If necessary, warm the solutions and mix well until the solutions become clear again.
- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes are capable of withstanding the centrifugal forces required.
- The spin columns provided with the Plasma cfDNA Extraction Kit are optimized to be used with benchtop centrifuges and not to be used on a vacuum apparatus.
- Centrifugation at a speed higher than recommended may affect DNA yield.
- Centrifugation at a speed lower than recommended will not affect DNA yield. However, it may require longer time for the solution to pass through the spin column.
- When placing Midi Columns into the swinging bucket centrifuge, make sure that lids of the tubes are NOT tightly closed during centrifugation. Tightly closed lids may cause back pressure and subsequent column clogging or disintegration.
- If any of the solutions do not go through the Spin Columns within the specified centrifugation time, spin for an additional 1-2 min. until the solution completely passes through the columns. Do NOT exceed the centrifugation speed as this may affect DNA yield.
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.

2. Extraction of Plasma cfDNA

*The procedure outlined below is for processing **1 ml to 4 ml of plasma**. If the sample volume is lower than 4 ml, simply bring the volume of your sample up to 4 ml using 1x PBS and proceed as outlined below.*
Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to DNA degradation. Frozen plasma (from blood collected on EDTA or Citrate tubes) should be centrifuged for 2 min. at 400 x g (2,000 rpm) before processing. Only clear supernatant should be processed, as column clogging may be encountered if frozen samples are directly processed.

- Place **4 ml plasma sample** in a 15 ml tube. Add **240 µl Proteinase K** and mix well by vortexing for 10 sec.
- Incubate for **20 min** at **60°C**.
- Add **7 ml Binding Buffer B**. Mix well by vortexing for 10 sec.
- Transfer **up to 5.5 ml of the resulting mixture** into a **Midi Column** assembled with one of the provided collection tubes. Centrifuge for **5 min.** at **2,000 x g (4,000 rpm)**. Discard the flow through and reassemble the spin column with its collection tube.
  ⚠ *Make sure that the lid of the tube is NOT tightly closed.*
- Repeat by transfering the **remaining mixture** into the Midi Column.
- Apply **1 ml Wash Buffer W1** to the **Midi Column**. Centrifuge for **5 min.** at **2,000 x g (4,000 rpm)**. Discard flow through and reassemble the Midi Column with its collection tube.
- Apply **1 ml Wash Buffer W2** to the **Midi Column**. Centrifuge for **5 min.** at **2,000 x g (4,000 rpm)**. Discard flow through and reassemble the Midi Column with its collection tube.
- Spin the Midi Column empty for **10 min.** at **2,000 x g (4,000 rpm)**. Discard the collection tube. Transfer the Midi Column to a fresh 15 ml tube.
- Apply **250 µl Elution Buffer E** to the **Midi Column** and let stand for **2 min.** at **room temperature**. Centrifuge for **5 min.** at **500 x g (1,600 rpm)**.
- Apply an additional **1 ml Elution Buffer E** to the **Midi Column** and let stand for **3 min.** at **room temperature**. Centrifuge for **5 min.** at **2,000 x g (4,000 rpm)**. At this point keep the flow through (Elution) and discard the Midi Column.
- To the Elution add **1.25 ml Binding Buffer B** and mix well by vortexing for 10 sec.
- Transfer **650 µl of this mixture** into a **Micro Column** assembled with one of the provided collection tubes. Centrifuge for **2 min.** at **3,300 x g (6,000 rpm)**. Discard the flow through and reassemble the Micro Column with its collection tube.
- Repeat this step three more times to transfer the **remaining mixture** into the Micro Column.
- Apply **500 µl Wash Buffer W1** to the **Micro Column** and centrifuge for **1 min.** at **3,300 x g (6,000 rpm)**. Discard the flow through and reassemble the Micro Column with its collection tube.
- Apply **500 µl Wash Buffer W2** to the **Micro Column** and centrifuge for **1 min.** at **3,300 x g (6,000 rpm)**. Discard the flow through and reassemble the Micro Column with its collection tube.
- Repeat this step one more time, for a total of two washes with Wash Buffer W2.
- Spin the Micro Column empty for **2 min.** at **13,000 x g (14,000 rpm)**. Discard the collection tube.
- Transfer the Micro Column to a fresh Elution tube. Apply **25-50 µl Elution Buffer E** to the **Micro Column** and let stand for **2 min.** at **room temperature**. Centrifuge for **1 min.** at **400 x g (2,000 rpm)**, followed by **2 min.** at **5,800 x g (8,000 rpm)**.

The resulting filtrate contains extracted plasma cfDNA suitable for various downstream applications. DNA should be kept frozen at -20°C.

Cell-free DNA (cfDNA) is normally found in very low amounts (1-100 pg/µl). Therefore measuring cfDNA concentration using common DNA quantification methods is very difficult and challenging. Typical yields of cfDNA vary significantly from sample to sample. Variability is also observed between samples collected from the same donor at different times during the day and therefore there is no absolute yield for cfDNA purified from bodily fluids including plasma. Cell-free DNA yield varies depending on a number of factors including age, sex, diet, exercise and most importantly the health status of the donor.

Optical density (OD) measurement at 260 nm or fluorescence-based quantification of cfDNA below 0.2 ng/µl is error-prone and hence may be inaccurate. The only reliable method that can assess the quality and the relative quantity of purified plasma cfDNA is ***qPCR amplification*** *of a standard DNA using a small DNA amplicon such as the 5S rRNA housekeeping gene.*

VI. QUALITY CONSIDERATIONS

- A thorough understanding of the procedure outlined here, and precise laboratory equipment and techniques are required to obtain reliable results.
- Do not use Plasma cfDNA Extraction Kit components beyond the expiration date printed on the outside of the kit box. Do not mix reagents from different lots.
- Avoid microbial contamination and cross-contamination of reagents or samples by using sterile disposable pipette tips throughout. Do not interchange bottle caps.

VII. SAFETY

- Do not drink, eat, smoke, or apply cosmetics in designated work areas. Wear laboratory coats and disposable gloves when handling specimens and kit reagents. Wash hands thoroughly afterwards.
- Handle specimens as if capable of transmitting infectious agents. Thoroughly clean and disinfect all materials and surfaces that have been in contact with specimens. Discard all waste associated with clinical specimens in a biohazard waste container.
- Avoid contact of Binding Buffer B, Proteinase K and Wash Buffer W1 with skin, eyes, or mucous membranes. If contact does occur, immediately wash with large amounts of water. If spilled, dilute with water before wiping dry.
- DO NOT add bleach or acidic solutions directly to the sample-preparation waste.
- Adhere to all local and federal safety and environmental regulations which may apply.

H302: Harmful if swallowed
H315: Causes skin irritation
H319: Causes serious eye irritation
H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled
H402: Harmful to aquatic life
P261: Avoid breathing vapor
P264: Wash hands thoroughly after handling
P273: Avoid release to the environment
P280: Wear protective gloves/protective clothing/eye protection/face protection
P301+312+330: If swallowed: Call a poison center or physician if you feel unwell. Rinse mouth
P302+352: If on skin: Wash with plenty of water
P332/337+313: If skin irritation occurs/persists: Get medical advice/attention

VIII. TROUBLESHOOTING

Advise on troubleshooting may be obtained by contacting ViennaLab through the local distributor or directly at the address provided on page I.

REF		Σ
2-014	GEN^{X}TRACT™ Blood DNA Extraction System	100 extractions
2-020	Spin Micro DNA Extraction Kit	20 extractions
2-030	D2PCR™ Buffer	100 extractions
2-040	Plasma cfDNA Extraction Kit	50 extractions

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