BioChain®
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User's Manual and Instructions

vamPure™ Blood Nucleic Acid Extraction Kit

Catalog Number: K5011720

Storage Conditions
This kit is shipped at room temperature. Upon receipt, store Proteinase K at -20°C. Store all remaining components of this kit at room temperature.

Shelf Life
1 year from the date of receipt under proper storage conditions

Features
- Non-toxic chemicals
- High DNA recovery
- Short and Scalable Protocol
- Purified DNA is suitable for NGS, PCR, Bisulfite sequencing, etc

Description
BioChain's vamPure™ Blood Nucleic Acid Extraction kit allows for fast and efficient genomic DNA extraction from whole blood samples. The DNA is eluted in EDTA-free buffer, allowing for immediate use in experiments where EDTA is not tolerated. EDTA may then be added separately for ideal long-term storage of the DNA. The magnetic bead-based extraction protocol is also ideally suited for automation using the KingFisher instrument. DNA extracted using this kit is suitable for PCR, qPCR, next generation sequencing (NGS), and other applications.

KingFisher Automation
The vamPure™ Blood Nucleic Acid Extraction Kit can be used to isolate DNA from up to 96, 200 µl Blood samples in one hour using the KingFisher™ Flex Magnetic Processor with 96 Deep Well Head. An alternative protocol below describes the use of the vamPure™ kit with the KingFisher™ Flex Magnetic Processor 96DW to process 200 µl blood samples.

Contents
This kit contains all necessary reagents for the isolation of genomic DNA from up to 20mls of whole blood sample(s).

Quality Control
Each component has been tested for purity and efficacy.

Important Notes
Starting Material: Both fresh and frozen whole blood can be used with the DNA isolation protocol.

Quantification: Depending on the donor, this kit will yield up to 15 ug of purified genomic DNA from a 200 µl sample of whole blood.

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Equipment and Reagents to be Supplied by User

- Pipettes
- Vortex-Genie 2 or similar vortexing mixer*
- Magnet stand for molecular applications (e.g. DynaMag™-15 or DynaMag™-2)
- 1.5 ml non-stick Eppendorf tubes
- 15 ml conical tubes
- 100% EtOH (200 proof)
- Isopropanol

* Contact BioChain® Technical Service for additional recommendations for high throughput or automated mixing.

Prior to Initial Use

BFC, FCW1, and FCW2 Buffers are shipped as a concentrate. If precipitate is present in any of these solutions, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

BFC Buffer

- Add 26.4 ml of isopropanol and mix by inverting gently

FCW1 Buffer

- Add 10.2 ml of 100% ethanol (200 proof) and mix by inverting gently

FCW2 Buffer

- Add 52.8 ml of 100% ethanol (200 proof) and mix by inverting gently

Once these alcohols are added, these buffers are stable for one year if stored properly. Be sure to close the bottle tightly for long term storage.

The protocol below was written assuming an initial sample volume of 200 µl. Any amount from 200 µl to 1 ml of whole blood can be used. Scale buffer and bead volumes accordingly (e.g. if 200 µl of blood requires 5 µl of beads, then 1 ml of blood requires 25 µl of beads, etc.). Also, use a 15 ml conical tube if using more than 350 ul of blood.

Protocol

Lysis/Binding

1. Add 200 µl of whole blood to a 1.5 ml Eppendorf tube
2. Add 200 µl of **GFC Buffer**
3. Add 10 ul of **Proteinase K**
4. Invert tube 5 times to mix and incubate at 56^{o}C for 5 minutes
5. Invert tube 5 times to mix and incubate at 56^{o}C for an additional 5 minutes
6. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube and add 300 µl of **BFC buffer**
7. Add 5 µl of **Magnetic Bead Solution**

   Important: Mix beads well prior to adding. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to every few samples. Failure to do so may result in inconsistent yields
8. Vortex vigorously for 10 minutes at room temperature

   * If a 20% reduction in yield is acceptable, this step may be shortened to 30 seconds. A vortexing mixer with a tube(s)-holder that allows for walk-away mixing will make this step easier.
9. Place tube onto a magnet stand and let sit for 3 minutes
10. While keeping the tube on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
11. Tap magnet stand on bench 5 times and remove remaining supernatant

Wash Steps

12. Transfer tube to non-magnetic rack and add 200 µl of **FCW1 Buffer**
    Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
    *Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is recommended to remove solution from tube lid. ALWAYS check to make sure the beads are at the bottom of the tube before spinning so that they don't become stuck on the side of the tubes and dry out. If beads are attached to the sides, flick the tube until beads are in solution
13. Allow beads to attach to magnet stand for 10-30 seconds
14. Remove as much supernatant as possible
15. Transfer tube to non-magnetic rack and add 200 µl of **FCW2 Buffer**
16. Pipet up and down several times and transfer bead solution to fresh 1.5 ml tube
    Important: This bead transfer step is important for obtaining consistently optimal A260/230 ratios. If the beads clump, use a 1000 µl tip and push down on the beads for very easy transfer
17. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
18. Allow beads to attach to magnet stand for 10-30 seconds
19. Remove as much supernatant as possible
20. Transfer tube to non-magnetic rack and add 200 µl of **FCW2 Buffer**
21. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
22. Allow beads to attach to magnet stand for 10-30 seconds
23. Remove as much supernatant as possible
24. Repeat steps 20-22 above once
25. Tap magnet stand on bench 5 times and remove residual supernatant as much as possible
26. Allow beads to dry for 3 minutes

Elution Step

27. Transfer tube to non-magnetic rack and add 150 µl of **FCE Buffer**
    Important:: A minimum of 50 µl of FCE Buffer is recommended to elute DNA to ensure optimal yields
28. Vortex vigorously for 10-15 seconds to loosen bead clumps
29. Incubate at 72^{o}C for 5 minutes
30. Vortex vigorously for 10-15 seconds to loosen any remaining bead clumps
31. Spin tube briefly
32. Place tube on magnetic rack for 3 minutes
33. Transfer eluate* into a new 1.5 ml Eppendorf tube

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*** Note:** To maximize the utilization of the final product in downstream applications, the Elution Buffer does not contain EDTA. If long-term storage of the DNA is desired, we recommend adding EDTA to a final volume of 1 mM (e.g. add 1 ul of 100 mM EDTA pH 8.0 stock solution for every 100 ul of elution volume).

200ul Sample KingFisher Flex Protocol

The vamPure™ Blood Nucleic Acid Extraction Kit can be used to isolate DNA from up to 96, 200 µl Blood samples using the KingFisher™ Flex Magnetic Processor with 96 Deep Well Head. This guide describes the use of the vamPure™ kit with the KingFisher™ Flex Magnetic Processor 96DW to process 200 µl blood samples.

Prior to Initial Use

BFC, FCW1, and FCW2 Buffers are shipped as a concentrate. If precipitate is present in any of the solutions, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

- **BFC Buffer**

  Add 26.4 ml of isopropanol and mix by inverting gently

- **FCW1 Buffer**

  Add 10.2 ml of 100% ethanol (200 proof) and mix by inverting gently

- **FCW2 Buffer**

  Add 52.8 ml of 100% ethanol (200 proof) and mix by inverting gently

Download KingFisher™ Flex Program

1. Visit vamPure™ Webpage scroll down to Manual Section.
2. Click vamPure_200ul_Flex to download program to your computer
3. Refer to KingFisher™ Flex manual for instructions on installing program on the instrument

Plate Set up

Set up 96 Plates by adding appropriate reagents according to table below

Plate ID	Plate Type	Plate Position on Instrument	Reagent	Volume per well
Lysis/Binding Plate	96 Deep-Well Plate	1	Blood Sample	200 µl
			GFC Buffer	200 µl
			Proteinase K	10 µl
Wash Plate 1	96 Deep-Well Plate	2	FCW1 Buffer	200 µl
Wash Plate 2	96 Deep-Well Plate	3	FCW2 Buffer	200 µl
Wash Plate 3	96 Deep-Well Plate	4	FCW2 Buffer	200 µl
Wash Plate 4	96 Deep-Well Plate	5	FCW2 Buffer	200 µl
Elution Plate	96 Standard Plate	6	FCE Buffer	150 µl
Tip Comb	96 Deep-Well Plate	7	Place a 96 Deep-Well Tip Comb in Plate	

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Instrument Set up

- Place 96 well Deep-Well magnetic head on to machine, and select vamPure_200ul_Flex on the instrument
- Start the run and follow on screen prompts to load processing plates in their respective positions
- 20 minutes after run starts a dispense step will prompt the user to add BFC Buffer and Magnetic Bead solution to Lysis/Binding Plate

Reagent	Volume per Well
BFC Buffer	300 µl
Magnetic Bead Solution	5 µl

- Place plate back into machine and press Start to continue protocol
- After program run ends isolated DNA is ready for immediate use

*** Note:** To maximize the utilization of the final product in downstream applications, the Elution Buffer does not contain EDTA. If long-term storage of the DNA is desired, we recommend adding EDTA to a final volume of 1 mM (e.g. add 1 ul of 100 mM EDTA pH 8.0 stock solution for every 100 ul of elution volume).

Kit Components

Item	Cat#	Amount	Storage
1. GFC Buffer	K5011720-1	20 ml	Room Temp
2. BFC Buffer	K5011720-2	6 ml	Room Temp
3. Proteinase K	K5011720-3	1 ml	-20°C
4. Magnetic Bead Solution	K5011720-4	500 µl	Room Temp
5. FCW1 Buffer	K5011720-5	10 ml	Room Temp
6. FCW2 Buffer	K5011720-6	12 ml	Room Temp
7. FCE Buffer	K5011720-7	15 ml	Room Temp