BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

vamPure™ Viral Nucleic Acid Extraction Kit

Catalog Number: K5011820

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 nucleic acid recovery
- Short and Scalable Protocol
- Purified nucleic acid is suitable for NGS, PCR, RT-PCR, Bisulfite sequencing, etc

Description
BioChain's vamPure™ Viral Nucleic Acid Extraction kit allows for fast and efficient nucleic acid extraction from swab samples. The nucleic acids are 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 nucleic acids. The magnetic bead-based extraction protocol is also ideally suited for automation using the KingFisher instrument. Nucleic acid extracted using this kit is suitable for PCR, qPCR, qRT-PCR, next generation sequencing (NGS), and other applications.

KingFisher Automation
The vamPure™ Viral Nucleic Acid Extraction Kit can be used to isolate total nucleic acid from up to 96, 200 µl swab 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 swab samples.

Contents
This kit contains all necessary reagents for the isolation of total nucleic acid from up to 20mls of swab sample(s).

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

Important Notes
Starting Material: Swab samples can be used with the isolation protocol.

Quantification: Due to the relatively low yields from swab samples, sensitive PCR methods are recommended for quantification.

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

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 sample can be used. Scale buffer and bead volumes accordingly (e.g. if 200 µl of sample requires 5 µl of beads, then 1 ml of sample requires 25 µl of beads, etc.). Also, use a 15 ml conical tube if using more than 350 ul of sample.

Protocol

Lysis/Binding

1. Add 200 µl of swab sample to a 1.5 ml Eppendorf tube
2. Add 200 µl of **GFC Buffer**

   * If the kit used to collect the swab already pre-lyses the sample, then this step may be omitted.
3. Add 10 ul of **Proteinase K**
4. Invert tube 5 times to mix and incubate at 56°C for 10 minutes
5. Invert tube 5 times to mix and incubate at 56°C for an additional 10 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

   * 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. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
17. Allow beads to attach to magnet stand for 10-30 seconds
18. Remove as much supernatant as possible
19. Transfer tube to non-magnetic rack and add 200 µl of **FCW2 Buffer**
20. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
21. Allow beads to attach to magnet stand for 10-30 seconds
22. Remove as much supernatant as possible
23. Tap magnet stand on bench 5 times and remove residual supernatant as much as possible
24. Allow beads to dry for 3 minutes

Elution Step

25. Transfer tube to non-magnetic rack and add 100 µl of **FCE Buffer**
    Important:: A minimum of 50 µl of FCE Buffer is recommended to elute nucleic acid to ensure optimal yields
26. Vortex vigorously for 10-15 seconds to loosen bead clumps
27. Incubate at 72°C for 5 minutes
28. Vortex vigorously for 10-15 seconds to loosen any remaining bead clumps
29. Spin tube briefly
30. Place tube on magnetic rack for 3 minutes
31. Transfer eluate* into a new 1.5 ml Eppendorf tube

*** 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 nucleic acid 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).

Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

200ul Sample KingFisher Flex Protocol

The vamPure™ Viral Nucleic Acid Extraction Kit can be used to isolate total nucleic acid from up to 96, 200 µl swab 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 swab 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_viral_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	Swab 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
Elution Plate	96 Deep-Well Plate	5	FCE Buffer	100 µl
Tip Comb	96 Deep-Well Plate	6	Place a 96 Deep-Well Tip Comb in Plate	

* If the kit used to collect the swab already pre-lyses the sample, then this buffer may be omitted.

Instrument Set up

- Place 96 well Deep-Well magnetic head on to machine, and select vamPure_viral_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 nucleic acid 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 nucleic acid 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	K5011820-1	20 ml	Room Temp
2. BFC Buffer	K5011820-2	6 ml	Room Temp
3. Proteinase K	K5011820-3	1 ml	-20°C
4. Magnetic Bead Solution	K5011820-4	500 µl	Room Temp
5. FCW1 Buffer	K5011820-5	10 ml	Room Temp
6. FCW2 Buffer	K5011820-6	12 ml	Room Temp
7. FCE Buffer	K5011820-7	15 ml	Room Temp