BioChain®
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User's Manual and Instructions

xTractPure™ Blood Nucleic Acid Extraction Kit

Catalog Number: Z2212001

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 xTractPure™ 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 BioChain's AnaPrep 48 instrument. DNA extracted using this kit is suitable for PCR, qPCR, next generation sequencing (NGS), and other applications.

AnaPrep 48 Automation
The xTractPure™ Blood Nucleic Acid Extraction Kit can be used to isolate DNA from up to 48, 200 µl Blood samples in one hour using the AnaPrep 48 instrument from BioChain (*plates and tip combs need to be ordered separately; please see catalog number Z2212099*). Enough reagents are included in each kit to process up to 96, 200 µl blood samples. An alternative protocol below describes the use of the xTractPure™ kit with the AnaPrep 48 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

- **96-well Deep Well plates and 8-strip Tip Combs (if using the AnaPrep 48 only)***
  **** BioChain Catalog Number: Z2212000***
- 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
- RNase A (optional)

* 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 25.5 ml of 100% ethanol (200 proof) and mix by inverting gently

FCW2 Buffer

- Add 88 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 10 µl of beads, then 1 ml of blood requires 50 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 µl of **Proteinase K**
4. Invert tube 5 times to mix and incubate at 56°C for 5 minutes
5. Invert tube 5 times to mix and incubate at 56°C for an additional 5 minutes
6. (Optional) Add 10 µl of RNase A (20 mg/ml), pipet up and down briefly to mix, and let incubate at room temperature for 5 minutes
7. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube and add 300 µl of **BFC buffer**
8. Add 10 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
9. 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.
10. Place tube onto a magnet stand and let sit for 3 minutes
11. While keeping the tube on the magnet stand, remove supernatant. Be careful not to remove magnetic particles

12. Tap magnet stand on bench 5 times and remove remaining supernatant

Wash Steps

13. Transfer tube to non-magnetic rack and add 500 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
14. Allow beads to attach to magnet stand for 10-30 seconds
15. Remove as much supernatant as possible
16. Transfer tube to non-magnetic rack and add 500 µl of **FCW2 Buffer**
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 500 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. Tap magnet stand on bench 5 times and remove residual supernatant as much as possible
25. Allow beads to dry for 3 minutes

Elution Step

26. Transfer tube to non-magnetic rack and add 50 µl of **FCE Buffer**
    Important:: A minimum of 50 µl of FCE Buffer is recommended to elute DNA to ensure optimal yields
27. Vortex vigorously for 10-15 seconds to loosen bead clumps
28. Incubate at 60°C for 10 minutes
29. Vortex vigorously for 10-15 seconds to loosen any remaining bead clumps
30. Spin tube briefly
31. Place tube on magnetic rack for 3 minutes
32. 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 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).

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200ul Sample AnaPrep 48 Protocol

The xTractPure™ Blood Nucleic Acid Extraction Kit can be used to isolate DNA from up to 96, 200 µl Blood samples using the AnaPrep 48 instrument with 96-well Deep Well Plates and 8-strip Tip Combs. Each Deep Well Plate can be used to extract DNA from up to 16 samples, allowing for higher throughput processing with minimal usage of consumable plastics. This guide describes the use of the kit with the AnaPrep 48 to process 200 µl blood samples.

Additional Materials Required

RNase A (optional)
Deep Well Plates and Tip Combs (Catalog No. Z2212000)

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

- **FCW2 Buffer**

  Add 88 ml of 100% ethanol (200 proof) and mix by inverting gently

Plate Set up

Set up 96-well Deep Well Plates by adding appropriate reagents according to table below

Each plate can accommodate 16 samples. If processing 8 or less samples on a plate, ensure that the reagents are added only to columns 1-6 or 7-12 on the plate. In other words, if FCW1 is added to column 2, then FCW2 buffers should be added to columns 3 and 4, not 9 and 10.

Step	Column Position on Plate	Reagent	Volume per well
Lysis/Binding	1, 7	Blood Sample	200 µl
		GFC Buffer	200 µl
		Proteinase K	10 µl
Wash 1	2, 8	FCW1 Buffer	500 µl
Wash 2	3, 9	FCW2 Buffer	500 µl
Wash 3	4, 10	FCW2 Buffer	500 µl
Elution	6, 12	FCE Buffer	50 µl*

*** Important:** A minimum of 50 µl must be present within the well of the plate in order for the elution step to take place.

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Instrument Set up

- Place 96-well Deep Well plate(s) into the instrument, ensuring that the heat strips are aligned with columns 1, 6, 7, and 12 of the plate
- Load 8-strip tip comb(s) into holder inside the instrument
- Touch the "**Sample Lysis**" button and then touch Continue
- The run will begin and end after 10 min
- Carefully remove the plate(s) from the instrument
- Optional: add 10 µl of RNase A (20 mg/ml) to each sample, pipet up and down briefly to mix, and let the plate sit at room temperature for 5 minutes
- Add BFC Buffer and Magnetic Bead solution to each sample according to the following table:

Reagent	Volume per Well
BFC Buffer	300 µl
Magnetic Bead Solution	10 µl

- Place plate(s) back into machine and touch the "**Blood DNA**" button
- Touch Continue to start the program
- After the run is complete, carefully remove plate(s) from the instrument
- Transfer the eluates (columns 6 and 12) from the plate to fresh tubes
- 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	Z2212001-1	20 ml	Room Temp
2. BFC Buffer	Z2212001-2	6 ml	Room Temp
3. Proteinase K	Z2212001-3	1 ml	-20°C
4. Magnetic Bead Solution	Z2212001-4	1 ml	Room Temp
5. FCW1 Buffer	Z2212001-5	25 ml	Room Temp
6. FCW2 Buffer	Z2212001-6	20 ml	Room Temp
7. FCE Buffer	Z2212001-7	15 ml	Room Temp

Appendix: Blood Nucleic Acid Program Settings for AnaPrep 48

The following shows the AnaPrep 48 program settings for the xTractPure™ Blood Nucleic Acid Extraction Kit.

Sample Lysis

Information of this step				Temperature Control			Mix		Waiting time		Magnetic binding	
Step	Well	Volume	Mode	Setup	Temp (°C)	Wait	Speed	Time (min)	Mode	Time (min)	Precipitate (sec)	Repeat (times)
Lysis	1	410	I	Lysis	60	N	Fast	10	0	0	1	0
End	1	50	End	Stop	0	N	Stop	0	0	0	1	1

Blood DNA

Information of this step				Temperature Control			Mix		Waiting time		Magnetic binding	
Step	Well	Volume	Mode	Setup	Temp (°C)	Wait	Speed	Time (min)	Mode	Time (min)	Precipitate (sec)	Repeat (times)
Bind	1	720	IV	Stop	0	N	Fast	10	0	0	20	1
Wash 1	2	500	IV	Stop	0	N	Fast	2	0	0	20	1
Wash 2	3	500	IV	Stop	0	N	Fast	2	0	0	20	1
Wash 3	4	500	IV	Stop	0	N	Fast	2	0	0	20	1
Elute	6	50	IV	Elute	60	N	Fast	10	0	3	30	3
End	1	50	End	Stop	0	N	Stop	0	0	0	1	1