BioChain®
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User's Manual and Instructions

xTractPure™ Cell and Tissue Nucleic Acid Extraction Kit

Catalog Number: Z2212005

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™ Cell and Tissue Nucleic Acid Extraction kit allows for fast and efficient genomic DNA extraction from cultured cell and biological tissue 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™ Cell and Tissue Nucleic Acid Extraction Kit can be used to isolate DNA from up to 48 cell pellets and/or tissue samples in under 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 cell pellet and/or tissue samples. An alternative protocol below describes the use of the xTractPure™ kit with the AnaPrep 48 to process samples.

Contents
This kit contains all necessary reagents for the isolation of genomic DNA from up to 96 cell pellet and/or tissue samples.

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

Important Notes
Starting Material (Cell Pellet):

Up to 1 x 10^6 of either fresh or frozen cell pellets may be used

Starting Material (Tissue):

For high-yield tissues (e.g. spleen), use up to 10 mg of tissue per extraction
For lower-yield tissues (e.g. liver), use up to 20 mg of tissue per extraction

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Quantification: Depending on the sample type, this kit will yield up to 20 ug of purified genomic DNA in a single extraction

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: Z2212099***
- 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.

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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.

Manual Protocol

Sample Preparation (Cell Pellet)

1. Take up to 1 x 10^6 fresh or frozen cells in a 1.5 ml Eppendorf tube and ensure media has been removed as much as possible
2. Add 400 µl of **CTFC Buffer** to the tube containing the cells
3. Add 10 µl of **Proteinase K**
4. Briefly vortex to mix, pipet up and down 10 times, and incubate at 56°C for 10 minutes
5. Vortex briefly 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
7. (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
8. Proceed to **Binding** steps in protocol

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Sample Preparation (Tissue)

1. For high-yield tissues (e.g. spleen), take up to 10 mg of tissue. For lower-yield tissues (e.g. liver), take up to 20 mg of tissue. For maximum yield, mince or grind the tissue into several small pieces
2. In a 1.5 ml Eppendorf tube, add 400 µl of **CTFC Buffer.** Then add 10 µl of **Proteinase K** and pipet up and down briefly to mix
3. Transfer the fresh or frozen tissue to the above tube
4. Place tube into either a thermal mixer or water bath and incubate at 56°C for 1-4 hours until all of the tissue pieces are dissolved

   If using a thermal mixer: shake continuously at 1,400 rpm during incubation

   If using a water bath: vortex the samples briefly every 10-15 minutes
5. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube
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. Proceed to **Binding** steps in protocol

Binding

1. To the sample tube, add 300 µl of **BFC buffer**
2. 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
3. Vortex vigorously for 10 minutes at room temperature
4. Place tube onto a magnet stand and let sit for 3 minutes
5. While keeping the tube on the magnet stand, remove supernatant. Be careful not to remove magnetic particles
6. Tap magnet stand on bench 5 times and remove remaining supernatant

Wash Steps

7. 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

8. Allow beads to attach to magnet stand for 10-30 seconds
9. Remove as much supernatant as possible
10. Transfer tube to non-magnetic rack and add 500 µl of **FCW2 Buffer**
11. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
12. Allow beads to attach to magnet stand for 10-30 seconds
13. Remove as much supernatant as possible
14. Transfer tube to non-magnetic rack and add 500 µl of **FCW2 Buffer**
15. Resuspend beads by vortexing for 15 seconds. Spin tube briefly and transfer back to magnet stand
16. Allow beads to attach to magnet stand for 10-30 seconds
17. Remove as much supernatant as possible
18. Tap magnet stand on bench 5 times and remove residual supernatant as much as possible
19. Allow beads to dry for 3 minutes

Elution Step

20. 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
21. Vortex vigorously for 10-15 seconds to loosen bead clumps
22. Incubate at 50°C for 10 minutes
23. Vortex vigorously for 10-15 seconds to loosen any remaining bead clumps
24. Spin tube briefly
25. Place tube on magnetic rack for 3 minutes
26. 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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AnaPrep 48 Protocol

The xTractPure™ Cell and Tissue Nucleic Acid Extraction Kit can be used to isolate DNA from up to 96 cell pellet and/or tissue 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. The instrument can be loaded with 3 Deep Well plates, thus allowing for simultaneous processing of up to 48 samples. This guide describes the use of the kit with the AnaPrep 48 to process cell and tissue samples.

Additional Materials Required

RNase A (optional)
1 package of Deep Well Plates and Tip Combs (Catalog No. Z2212099)

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

Sample Preparation (Cell Pellet)

1. Take up to 1 x 10^6 fresh or frozen cells in a 1.5 ml Eppendorf tube and ensure media has been removed as much as possible
2. Add 400 µl of **CTFC Buffer** to the tube containing the cells
3. Add 10 µl of **Proteinase K**
4. Briefly vortex to mix, pipet up and down 10 times, and incubate at 56°C for 10 minutes
5. Vortex briefly to mix and incubate at 56°C for an additional 10 minutes
   - During this incubation period, set up 96-well Deep Well plates with appropriate reagents as shown in **Plate Set Up** section
6. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube
7. (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
8. Spin tube briefly to pellet any residual cell debris. Transfer resulting supernatant (~400 µl) to the **Binding** position of the 96-well Deep Well plate

Sample Preparation (Tissue)

1. For high-yield tissues (e.g. spleen), take up to 10 mg of tissue. For lower-yield tissues (e.g. liver), take up to 20 mg of tissue. For maximum yield, mince or grind the tissue into several small pieces
2. In a 1.5 ml Eppendorf tube, add 400 µl of **CTFC Buffer.** Then add 10 µl of **Proteinase K** and pipet up and down briefly to mix
3. Transfer the fresh or frozen tissue to the above tube
4. Place tube into either a thermal mixer or water bath and incubate at 56°C for 1-4 hours until all of the tissue pieces are dissolved

   If using a thermal mixer: shake continuously at 1,400 rpm during incubation

   If using a water bath: vortex the samples briefly every 10-15 minutes

   - A few minutes before the incubation period is complete, set up 96-well Deep Well plates with appropriate reagents as shown in **Plate Set Up**

5. Spin tube briefly on a tabletop centrifuge to bring down solution from the lid of the tube
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 to pellet any residual tissue debris. Transfer resulting supernatant (~400 µl) to the **Binding** position of the 96-well deep-well plate

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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
Binding	1, 7	Cell or Tissue Sample	400 µl
		BFC Buffer	300 µl
		Magnetic Bead Solution	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.

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 "**CellTissueDNA**" button and then touch Continue to begin the run
- 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).

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Kit Components

Item	Cat#	Amount	Storage
1. CTFC Buffer	Z2212005-1	40 ml	Room Temp
2. BFC Buffer	Z2212005-2	6 ml	Room Temp
3. Proteinase K	Z2212005-3	1 ml	-20°C
4. Magnetic Bead Solution	Z2212005-4	1 ml	Room Temp
5. FCW1 Buffer	Z2212005-5	25 ml	Room Temp
6. FCW2 Buffer	Z2212005-6	20 ml	Room Temp
7. FCE Buffer	Z2212005-7	15 ml	Room Temp

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Appendix: Cell and Tissue Nucleic Acid Program Settings for AnaPrep 48

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

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	710	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	50	N	Fast	10	0	3	30	3
End	1	50	End	Stop	0	N	Stop	0	0	0	1	1