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

User's Manual and Instructions

xTractPure™ FFPE DNA Extraction Kit

Catalog Number: **Z2212002**

Shipping Condition: Shipped at room temperature.

Storage Condition
Upon receipt, store Proteinase K at -20°C. Store the remaining contents at room temperature.

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

Introduction:
Formalin-fixed, paraffin-embedded (FFPE) tissue specimens are highly valuable sources for retrospective studies of many pathologies. Nevertheless, the extraction of nucleic acids from FFPE specimens could often be challenging, as nucleic acids become cross-linked and degraded during the archiving process. Therefore, nucleic acids obtained from these sample types are usually highly fragmented and chemically modified.

Description
BioChain's xTractPure™ FFPE DNA Extraction Kit allows for efficient DNA extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qPCR and NGS. Utilizing heat and proteinase K treatment, BioChain's kit is optimized in the removal of paraffin, partial reversal of formalin cross-linking, and release of DNA from fixed tissues.

Features
- No toxic chemicals
- No loss of nucleic acids
- Robust protocol suitable for automation
- No inhibition on downstream applications

Content
All necessary reagents for DNA extractions in FFPE tissue specimens are provided. The kit contains sufficient reagents for 48 FFPE DNA extractions.

Quality Control
All kit components are DNase-, RNase-, and protease-free. Each component has been tested for purity and efficacy.

Important Notes
Starting Material: The starting tissue material shall be freshly cut FFPE tissue sections with thickness of up to 10 µm each with surface area of up to 200 mm^2 for each 200 µl reaction. The extraction protocols and reagents are easily scalable to accommodate larger or smaller amount of input sections.

Recommendations for downstream PCR applications: Due to the highly fragmented nature of the nucleic acids obtained from FFPE tissues, particular care should be taken in the design of primers. PCR amplification shall be less than 300 bases in length with PCR profiles at 40 amplification cycles to ensure successful amplification.

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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)
- RNase A (optional)

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

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Prior to Initial Use

If precipitate is present in either the FFPE DNA Lysis Buffer or Wash Buffer 1, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

- Add 13.5 ml of 100% Ethanol to the bottle of Wash Buffer 1 and mix well.
- Add 38.5 ml of 100% Ethanol to the bottle of Wash Buffer 2 and mix well.

Protocol

FFPE DNA Lysis/Binding

1. Cut sections 6-10 µm thick
2. Place tissue sections directly into a 1.5 ml Eppendorf tube
3. Add **Dewaxil** to the sample
   • For 4-8 sections, add 500 µl of Dewaxil
4. Incubate at 90°C for 2 minutes. Vortex to mix
5. Add 200 µl of **FFPE DNA Lysis Buffer** into each sample tube
6. Vortex briefly to mix and spin at 10,000 x g for 1 minute at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.
7. Add 20 µl of **Proteinase K** directly to the lower phase; mix the lower phase by pipetting up and down 20 times
8. Incubate tube at 56°C for at least 1 hour with intermittent mixing (shaker/rotator preferred)
9. Incubate specimen samples at 90°C for 1 hour without mixing
10. Cool down the tube for a couple of minutes at room temperature
11. Centrifuge the tube at 10,000 x g for 2 minutes at room temperature
12. Carefully transfer the bottom layer excluding any tissue residue to a new 1.5 ml Eppendorf tube, avoiding white paraffin residues
13. (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
14. Add 200 µl of **Binding Buffer** and 400ul of **100% Ethanol**

15. Add 20 µl of **Magnetic Beads** and vortex at RT for 10 min

    Important: Mix beads well by vortexing 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

16. Place tube onto a magnetic rack and let sit until the beads have completely separated from the solution
17. Aspirate and discard the clear supernatant. Do not disturb the beads particles

FFPE DNA Wash Steps

18. Remove the tube from the magnetic rack. Add 500 µl of **Wash Buffer 1** to the tube and resuspend the beads particles by vortexing for 10 seconds
19. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution
20. Aspirate and discard the clear supernatant. Do not disturb the beads
21. Remove the tube from the magnetic rack. Add 500 µl of **Wash Buffer 2** to the tube and resuspend the beads particles by vortexing for 10 seconds
22. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution
23. Aspirate and discard the clear supernatant. Do not disturb the beads
24. Repeat steps 16-18 above once
25. Tap magnetic rack on bench 5 times and remove residual supernatant as completely as possible
26. Allow beads to dry for 3 minutes

FFPE DNA Elution Step

27. Remove the tube from the magnetic rack. Add 50 µl of **Elution Buffer** to the tube and resuspend the beads particles by vortexing for 10 seconds
28. Incubate at 60°C for 10 minutes
29. Spin tube briefly
30. Place tube onto a magnet stand and let sit for 3 minutes
31. Transfer eluate into a new 1.5 ml Eppendorf tube
32. Check the concentration either by UV or pico green, and store the DNA at -20°C (BioChain recommends pico green measurement for more accurate FFPE DNA concentration)

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AnaPrep 48 Protocol for Automated Extraction

Description
BioChain's xTractPure™ FFPE DNA Extraction Kit allows for efficient DNA extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qPCR and NGS. This kit may be applied on our AnaPrep 48 instrument for high-throughput extractions by automating the binding, wash and elution steps of the protocol.

Additional Materials Required

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

Prior to Initial Use

If precipitate is present in either the FFPE DNA Lysis Buffer or Wash Buffer 1, incubate solution at 37°C for 30 minutes with occasional shaking. Add the following user-supplied reagents to each bottle:

- Add 13.5 ml of 100% Ethanol to the bottle of Wash Buffer 1 and mix well.
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FFPE DNA Lysis/Binding

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3. Add **Dewaxil** to the sample
   • For 4-8 sections, add 500 µl of Dewaxil
4. Incubate at 90°C for 2 minutes. Vortex to mix
5. Add 200 µl of **FFPE DNA Lysis Buffer** into each sample tube
6. Briefly vortex to mix and spin at 10,000 x g for 1 minute at room temperature. Two phases will be formed, a lower (aqueous) phase and an upper (Dewaxil) phase.
7. Add 20 µl of **Proteinase K** directly to the lower phase; mix the lower phase by pipetting up and down 20 times
8. Incubate tube at 56°C for at least 1 hour with intermittent mixing (shaker/rotator preferred)
9. Incubate specimen samples at 90°C for 1 hour without mixing. During this time, set up 96-well plates as needed by following the Plate Setup procedure below
10. Cool down the tube for a couple of minutes at room temperature
11. Centrifuge the tube at 10,000 x g for 2 minutes at room temperature

12. (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

13. Carefully transfer the bottom layer excluding any tissue residue to the 96-well plate in column 1 and/or 7 based on the Plate Setup table

Plate Setup for FFPE DNA Extraction

Set up each 96-well Deep Well Plate by adding appropriate reagents according to table below. This may be done ahead of time during the 90°C incubation period. Each plate can accommodate 16 samples. If processing 8 or less samples, ensure that the reagents are added only to columns 1-6 or 7-12 on the plate. In other words, if Wash Buffer 1 is added to column 3, then Wash Buffer 2 should be added to columns 4 and 5, not 10 and 11.

Step	Column Position on Plate	Reagent	Volume per well
Binding	1, 7	Sample	200 µl
		Binding Buffer	200 µl
		100% Ethanol	400 µl
		Magnetic Beads	20 µl
Wash 1	2, 8	Wash Buffer 1	500 µl
Wash 2	3, 9	Wash Buffer 2	500 µl
Wash 3	4, 10	Wash Buffer 2	500 µl
Elution	6, 12	Elution 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.

14. Load plate(s) into AnaPrep 48 instrument, ensuring that the heat strips are aligned with columns 1, 6, 7, and 12 of the plate

15. Load 8-strip tip comb(s) into holder inside the instrument

16. Turn on instrument and tap the button "**FFPE DNA**" on the touch screen. Touch Continue to start

17. When the run is complete, remove the plate carefully from the instrument

18. Transfer eluates from column 6 and/or 12 to fresh tubes. Store the extracted FFPE DNA at -20°C

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Kit Components

Item	Part #	Amount	Storage
1. Dewaxil	Z2212002-1	26 ml	Room Temp
2. Proteinase K	Z2212002-2	1.1 ml	-20°C
3. Magnetic Beads	Z2212002-3	1.1 ml	Room Temp
4. FFPE DNA Lysis Buffer	Z2212002-4	11 ml	Room Temp
5. Binding Buffer	Z2212002-5	11 ml	Room Temp
6. Wash Buffer 1	Z2212002-6	13.5 ml	Room Temp
7. Wash Buffer 2	Z2212002-7	16.5 ml	Room Temp
8. Elution Buffer	Z2212002-8	2.8 ml	Room Temp

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Appendix: FFPE DNA Program Settings for AnaPrep 48

The following shows the AnaPrep 48 program settings for the xTractPure™ FFPE DNA Extraction Kit.

FFPE 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	820	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