BioChain®
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User's Manual and Instructions

xTractPure™ FFPE DR Extraction Kit

Catalog Number: **Z2212004**

Shipping Condition: Shipped at room temperature. DNase and DNase buffer shipped with dry ice (if purchased).

Storage Condition

Upon receipt, store Proteinase K at -20°C. Aliquot DNase, and DNase buffer into appropriate amounts and store at -20°C upon arrival. 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 DR Extraction Kit allows for efficient RNA and 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 sequential release of RNA and DNA from fixed tissues. One of the great advantages of our kit is that both FFPE RNA and FFPE DNA can be extracted separately from the same piece of tissue. This is critical for precious biological samples where source material is very limited, and it is not practical to have separate tissue sections for RNA and DNA extraction.

Features

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

Content

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

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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.

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)
- DNase

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

Protocol
Prior to initial use:

If precipitate is present in either the FFPE RNA Lysis Buffer, 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 27 ml of 100% Ethanol to the bottle of Wash Buffer 1 and mix well.
- Add 77 ml of 100% Ethanol to the bottle of Wash Buffer 2 and mix well.

FFPE RNA Extraction

FFPE RNA 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 RNA 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 15 minutes with mixing (shaker/rotator preferred)
9. Spin at 10,000 x g for 2 minutes at room temperature
10. Carefully transfer the bottom layer without disturbing the tissue pellet to a new 1.5 ml Eppendorf tube, avoiding white paraffin residues.
11. Incubate the transferred bottom layer at 80°C for 15 minutes. The remaining tissue pellet will be used to process FFPE DNA extraction (proceed to FFPE DNA Extraction section of this protocol)
    Note: The pellet with Dewaxil may be stored frozen at -80°C if FFPE DNA extraction will be performed at a later time.
12. Cool down the tube for a couple of minutes at room temperature and spin briefly
13. Add 200 µl of **Binding Buffer** and 400ul of **100% Ethanol**
14. 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

During this incubation step, prepare the following DNase mix and keep on ice:

Per sample

62 µl Nuclease-free water
8 µl 10x DNase buffer
10 µl DNase

15. Place tube onto a magnetic rack and let sit until the beads have completely separated from the solution

16. Aspirate and discard the clear supernatant. Do not disturb the beads

FFPE RNA Wash Steps

17. 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

18. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution

19. Aspirate and discard the clear supernatant. Do not disturb the beads particles

20. 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

21. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution

22. Aspirate and discard the clear supernatant. Do not disturb the beads particles

FFPE RNA DNase Treatment

23. Add the 80 µl of previously prepared **DNase mix** to the sample tube containing the beads

24. Vortex briefly and incubate at 37°C for 10 minutes with intermittent mixing

25. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution

26. Aspirate and discard the clear supernatant. Do not disturb the beads

FFPE RNA Wash Steps

27. 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

28. Place tube onto the magnetic rack and let sit until the beads have completely separated

from the solution

29. Aspirate and discard the clear supernatant. Do not disturb the beads particles
30. Repeat steps 27-29 above once
31. Tap magnetic rack on bench 5 times and remove residual supernatant as completely as possible
32. Allow beads to dry for 3 minutes

FFPE RNA Elution Step

33. Remove the tube from the magnetic rack. Add 50 µl of **Nuclease-free Water** to the tube and resuspend the beads particles by vortexing for 10 seconds
34. Incubate at 60°C for 10 minutes
35. Spin tube briefly
36. Place tube onto a magnet stand and let sit for 3 minutes
37. Transfer eluate into a new 1.5 ml Eppendorf tube and store the RNA at -80°C

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FFPE DNA Extraction

FFPE DNA Lysis/Binding

1. To the saved tissue pellet from the FFPE RNA Lysis/Binding protocol, add 200 µl of **FFPE DNA Lysis Buffer**
2. 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.
3. Add 20 µl of **Proteinase K** directly to the lower phase; mix the lower phase by pipetting up and down 20 times
4. Incubate tube at 56°C for at least 1 hour with intermittent mixing (shaker/rotator preferred)
5. Incubate specimen samples at 90°C for 1 hour without mixing
6. Cool down the tube for a couple of minutes at room temperature
7. Centrifuge the tube at 10,000 x g for 2 minutes at room temperature
8. Carefully transfer the bottom layer excluding any tissue residue to a new 1.5 ml Eppendorf tube, avoiding white paraffin residues
9. Add 200 µl of **Binding Buffer** and 400ul of **100% Ethanol**
10. 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

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

FFPE DNA Wash Steps

13. 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
14. Place tube onto the magnetic rack and let sit until the beads have completely separated from the solution
15. Aspirate and discard the clear supernatant. Do not disturb the beads
16. 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
17. Place tube onto the magnetic rack and let sit until the beads have completely separated

from the solution

18. Aspirate and discard the clear supernatant. Do not disturb the beads
19. Repeat steps 16-18 above once
20. Tap magnetic rack on bench 5 times and remove residual supernatant as completely as possible
21. Allow beads to dry for 3 minutes

FFPE DNA Elution Step

22. 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
23. Incubate at 60°C for 10 minutes
24. Spin tube briefly
25. Place tube onto a magnet stand and let sit for 3 minutes
26. Transfer eluate into a new 1.5 ml Eppendorf tube
27. 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 DR Extraction Kit allows for efficient RNA and 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, DNase-treatment, wash and elution steps of the protocol.

Additional Materials Required

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

Protocol

Prior to initial use:

If precipitate is present in either the FFPE RNA Lysis Buffer, 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 27 ml of 100% Ethanol to the bottle of Wash Buffer 1 and mix well.
- Add 77 ml of 100% Ethanol to the bottle of Wash Buffer 2 and mix well.

FFPE RNA Extraction

FFPE RNA 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 RNA 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 15 minutes with intermittent mixing (shaker/rotator preferred). During this step (or the subsequent incubation at step 11 below), set up 96-well plates as needed by following the Plate Setup procedure on the following page
9. Spin at 10,000 x g for 2 minutes at room temperature
10. Carefully transfer the bottom layer without disturbing the tissue pellet to a new 1.5 ml Eppendorf tube, avoiding white paraffin residues.

11. Incubate the transferred bottom layer at 80°C for 15 minutes. The remaining tissue pellet will be used to process FFPE DNA extraction (proceed to FFPE DNA Extraction section of this protocol)
    Note: The pellet with Dewaxil may be stored frozen at -80°C if FFPE DNA extraction will be performed at a later time.

12. Cool down the tube for a couple of minutes at room temperature and spin briefly

13. Transfer the sample to the 96-well plate in column 2 and/or 8 based on the Plate Setup table

Plate Setup for FFPE RNA 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 56°C and/or 80°C incubation periods. The DNase mix should be prepared according to the following formula and kept on ice until just before the samples are ready to be transferred to the plate:

Per sample

62 µl Nuclease-free water
8 µl 10x DNase buffer
10 µl DNase

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	2, 8	Sample	200 µl
		Binding Buffer	200 µl
		100% Ethanol	400 µl
		Magnetic Beads	20 µl
DNase Treatment	1, 7	DNase mix	80 µl
Wash 1	3, 9	Wash Buffer 1	500 µl
Wash 2	4, 10	Wash Buffer 2	500 µl
Wash 3	5, 11	Wash Buffer 2	500 µl
Elution	6, 12	Nuclease-free Water	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 RNA**" 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 RNA at -80°C

FFPE DNA Extraction

FFPE DNA Lysis/Binding

1. To the saved tissue pellet from the FFPE RNA Lysis/Binding protocol, add 200 µl of **FFPE DNA Lysis Buffer** (if sample was frozen, it may be warmed at 56°C for a couple of minutes until the Dewaxil becomes fluid)
2. 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.
3. Add 20 µl of **Proteinase K** directly to the lower phase; mix the lower phase by pipetting up and down 20 times
4. Incubate tube at 56°C for at least 1 hour with intermittent mixing (shaker/rotator preferred)
5. 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
6. Cool down the tube for a couple of minutes at room temperature
7. Centrifuge the tube at 10,000 x g for 2 minutes at room temperature
8. 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	1000 µl
Wash 2	3, 9	Wash Buffer 2	500 µl
Wash 3	4, 10	Wash Buffer 2	1000 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.

9. Load plate(s) into AnaPrep 48 instrument, ensuring that the heat strips are aligned with columns 1, 6, 7, and 12 of the plate
10. Load 8-strip tip comb(s) into holder inside the instrument
11. Turn on instrument and tap the button "**FFPE DNA**" on the touch screen. Touch Continue to start
12. When the run is complete, remove the plate carefully from the instrument
13. Transfer eluates from column 6 and/or 12 to fresh tubes. Store the extracted FFPE DNA at -20°C

Kit Components

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xTractPure™ FFPE DR Extraction Kit (Z2212004)

Item	Part #	Amount	Storage
1. Dewaxil	Z2212004-1	26 ml	Room Temp
2. Proteinase K	Z2212004-2	2 x 1.1 ml	-20°C
3. Magnetic Beads	Z2212004-3	2 x 1.1 ml	Room Temp
4. FFPE RNA Lysis Buffer	Z2212004-4	11 ml	Room Temp
5. FFPE DNA Lysis Buffer	Z2212004-5	11 ml	Room Temp
6. Binding Buffer	Z2212004-6	22 ml	Room Temp
7. Wash Buffer 1	Z2212004-7	27 ml	Room Temp
8. Wash Buffer 2	Z2212004-8	33 ml	Room Temp
9. Nuclease-free Water	Z2212004-9	6 ml	Room Temp
10. Elution Buffer	Z2212004-10	2.8 ml	Room Temp

xTractPure™ DNase-Treatment Box (Z2212099)*

Item	Part #	Amount	Storage
1. DNase Buffer	Z2212099-1	425 µl	-20°C
2. DNase	Z2212099-2	550 µl	-20°C

* This is an optional box. The customers may choose to provide their own alternative DNase and buffer

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Appendix: FFPE Nucleic Acid Program Settings for AnaPrep 48

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

FFPE RNA

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	2	820	IV	Stop	0	N	Fast	10	0	0	20	1
Wash 1	3	1000	IV	Stop	0	N	Fast	2	0	0	20	1
Wash 2	4	500	IV	Stop	0	N	Fast	2	0	0	20	1
DNase	1	80	IV	Lysis	37	N	Medium	10	0	0	20	1
Wash 3	5	1000	IV	Stop	0	N	Fast	2	0	0	20	1
Elute	6	50	IV	Elute	60	N	Fast	5	0	3	30	3
End	1	50	End	Stop	0	N	Stop	0	0	0	1	1

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