

User's Manual and Instructions

xTractPure™ FFPE RNA Extraction Kit

Catalog Number: Z2212003

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 RNA Extraction Kit allows for efficient RNA extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qRT-PCR 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 RNA from fixed tissues. The eluted FFPE RNA is high-quality and may be analyzed for DV200 readings in preparation for downstream applications such as spatial profiling of 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 RNA extractions in FFPE tissue specimens are provided. The kit contains sufficient reagents for 48 FFPE RNA 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 mm² 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.

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 (if not purchased from BioChain)

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

Prior to Initial Use

If precipitate is present in either the FFPE RNA 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 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.
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 μ l Nuclease-free water
8 μ l 10x DNase buffer
10 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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 μ l 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

AnaPrep 48 Protocol for Automated Extraction

Description

BioChain's xTractPure™ FFPE RNA Extraction Kit allows for efficient RNA extraction from FFPE tissues, with potential high throughput capabilities and full compatibility for down-stream applications such as qRT-PCR 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)

Prior to Initial Use

If precipitate is present in either the FFPE RNA 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.

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

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	1000 µl
Wash 2	4, 10	Wash Buffer 2	500 µl
Wash 3	5, 11	Wash Buffer 2	1000 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

Kit Components**xTractPure™ FFPE RNA Extraction Kit (Z2212003)**

Item	Part #	Amount	Storage
1. Dewaxil	Z2212003-1	26 ml	Room Temp
2. Proteinase K	Z2212003-2	1.1 ml	-20°C
3. Magnetic Beads	Z2212003-3	1.1 ml	Room Temp
4. FFPE RNA Lysis Buffer	Z2212003-4	11 ml	Room Temp
5. Binding Buffer	Z2212003-5	11 ml	Room Temp
6. Wash Buffer 1	Z2212003-6	13.5 ml	Room Temp
7. Wash Buffer 2	Z2212003-7	16.5 ml	Room Temp
8. Nuclease-free Water	Z2212003-8	6 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

Appendix: FFPE RNA Program Settings for AnaPrep 48

The following shows the AnaPrep 48 program settings for the xTractPure™ FFPE RNA 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