Content

- *BiOstic FFPE Tissue DNA and RNA Isolation Kit*
- *FFPE Histology Tissue Curl DNA/RNA Extraction – Manual Method*

(1) BiOstic FFPE Tissue DNA and RNA Isolation Kit

Modified from:

1) BiOstic FFPE tissue DNA isolation kit instruction manual
2) BiOstic FFPE tissue RNA isolation kit instruction manual

REMINDERS: When working with DNA/RNA, always wear fresh clean gloves and a labcoat. Clean all equipment/ working spaces with RNAse Away and/or 100% ethanol prior to use in order to remove contaminating nucleic acids and RNAses which may degrade samples.

DNA Isolation Kit

- **Total Time:** 3-5 hours

Equipment Needed:	**Reagents:**
1. Microcentrifuge (13000 x g) 2. Pipette tips and pipettes 3. Waterbath or heat block sets (preferably two heat blocks set at 55°C and 90°C) 4. Vortex machine	All reagents required for the DNA isolation kit are given and labeled as FP1 to FP8; the amount of reagents given is enough for 50 isolation preps.

BEFORE Starting:

1. All steps are carried out in room temperature
2. Dissolve precipitate in FP1 and FP2 (if formed) by warming at 37°C
3. Set up two heat blocks; one at 55°C and one at 90°C. If a second heat block is not available, then remove samples from 55°C block after incubation, then wait for the block to go up to 90°C before placing samples in again. DO NOT leave samples in the heat block as it heats up.

Tissue Processing

1. Add 180 uL of FP1 and 20 uL of FP2 to the tube containing the tissue curls. One curl is enough for adequate extraction and isolation of DNA. Vortex for 20 seconds, then centrifuge to bring down solution.
2. Add 20 uL of FP3, which is proteinase K. Vortex. Heat the sample at 55°C for a minimum of 1 hour. (**NOTE:** From experience, heating the sample for at least two hours gave better yields than just heating for one hour at this stage)
3. Transfer the samples to the heat block set to 90°C (**NOTE:** Note above before starting if only one heat block is available). It is **critical** to heat for only one hour at this step.

4. Centrufge the samples at 13,000 x g, for 1-2 minutes, and transfer the digested lysates to a new 2 mL collection tube. Add 200 uL of FP4, vortex to mix. Then, add 200 uL of FP5, vortex to mix.
5. Load entire lystate onto a **spin filter tube,** then centrifuge for 1-2 minutes at 10,000 g.
6. Remove the flow-through, then put the filter back into tube, and add 500 uL of FP6. Centrifuge 1-2 minutes at 10,000 g.
7. Place spin filter into a new collection tube, and 500 uL of FP7 and centrifuge 10,000 g for 1 minute. Discard flow through. Centrifuge for 2 minutes at full speeds to dry the membrane and then transfer again to a new spin tube.
8. Elute the purified DNA with 50-100 uL of FP8 (Tris pH 8.0, 10 mM). Note, the lower the volume of FP8 added, the greater the concentration of DNA when measuring. Although the instruction manual says 5 minutes at room temperature, **leaving it for 10 minutes may increase amount of DNA eluted**. Centrifuge at 1-2 minute for 10,000 g.
9. Final solution at the bottom of the collection tube is the product, which contains the DNA from the tissue curls.

Note: Consult instruction manual for more details; from experience, longer centrifuge times helps with having greater concentration of DNA.

RNA Isolation Kit

- **Total Time:** 4-6 hours

Equipment Needed:	**Reagents:**
1. Microcentrifuge (13,000 to 16,000 x g) 2. Pipettors 3. Water bath or heat block at 60°C 4. Water bath or heat block at 70°C 5. Vortex machine	6. 100% ethanol 7. The other reagents are provided in the kit, and contains solutions labeled FR1 to FR7. Additionally, RTS DNase is given.

BEFORE Starting:

1. Set up heat blocks in advance, one at 60°C and one at 70°C.
2. Store FR2 and RTS DNase at 4°C; store low elution spin filters at 4°C. All else can be stored at room temperature.
3. Wear RNase-free gloves and clean all equipment to prevent contamination.

Tissue Processing:

1. Add 300 uL of FR1 and 20 uL of FR2 to tube containing tissue curl. Only about 1-2 curls are needed to obtain adequate concentration. Vortex the tube for 20 seconds, and bring solution down by centrifuging at 2000 x g.
2. Heat the sample for 15 minutes at 60°C; transfer the sample to the 70°C for 15 minutes.

3. Centrifuge the samples at 13,000 x g for 1-2 minutes, then transfer the digested lysate to new 2 mL collection tube. Use a pipet to transfer this amount.
4. Add 300 uL of FR3, and 600 uL of 100% absolute ethanol. Mix by vortexing or pipetting. Load 600 uL to a low elution spin filter, centrifuge at 13,000 x g for 30-60 seconds.
5. Repeat step four (transfer remaining solution from collection tube into the spin filter).
6. Add 600 uL of solution FR4, centrifuge at 13,000 x g for 30-60 seconds. Decant, then add 600 uL of ethanol, centrifuge at 13,000 x g for 30-60 seconds. Decant flow-through, then dry spin the column in the centrifuge for 2 minutes at 16,000 x g.
7. Transfer the low elution spin filter to a clean 2 mL collection tube. Add 50 uL of the RTS DNase/FR5 (pre-mix 4 uL RTS DNase and 46 uL of FR5 per prep).
8. Add 400 uL of FR6 to the low elution spin filter, and centrifuge for 30 seconds at 13,000 x g.
9. Decant, then load 600 uL of FR4 to the spin filter, then centrifuge for 30 seconds at 13,000 x g. Decant, then load 600 uL of 100% ethanol. Centrifuge for 30 seconds at 13,000 x g.
10. Dry spin the column in the centrifuge for 2 minutes at 16,000 x g.
11. Discard the collection tube and transfer the spin filter to a new and clean collection tube.
12. Elute with 20 uL of FR7 for 10 minutes at room temperature to maximize RNA recovery. Centrifuge for 2 minutes at 16,000 x g. The eluted RNA will be in the collection tube, and throw away the spin filter. Store in -80°C until ready for use.

Note: Consult instruction manual for more details; from experience, longer centrifuge times helps with having greater concentration of DNA.

Examples of Good Yields

Sample ID	Tissue Type	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type
118	HL	0.694	µg/µl	17.35	8.359	2.08	2.01	RNA
118	HL	0.4866	µg/µl	9.733	4.948	1.97	2.17	DNA
1778	HL	0.2897	µg/µl	7.242	3.561	2.03	1.87	RNA
1778	HL	0.3233	µg/µl	6.465	3.259	1.98	2.01	DNA
2174	HL granuloma	0.788	µg/µl	19.7	9.941	1.98	1.52	RNA
2174	HL granuloma	1.084	µg/µl	21.679	10.808	2.01	2.17	DNA
12187	HL	0.8584	µg/µl	21.461	10.409	2.06	1.92	RNA
12187	HL	0.9578	µg/µl	19.156	9.39	2.04	2.22	DNA

(2) FFPE Histology Tissue Curl DNA/RNA Extraction- Manual Method

Modified from:

1) Loudig et al., Nucl. Acid Res. 2007, *Molecular restoration of archived transcriptional profiles by complementary-template reverse-transcription (CT-RT)*
2) Kotorashi et al., PLOS ONE 2012, *Effective DNA/RNA co-extraction for analysis of microRNAs, mRNAs, and genomic DNA from formalin-fixed paraffin-embedded specimens*
3) TRIZOL Reagent product information sheet protocol (Invitrogen)

REMINDERS: When working with DNA/RNA, always wear fresh clean gloves and a labcoat. Clean all equipment/ working spaces with RNAse Away and/or 100% ethanol prior to use in order to remove contaminating nucleic acids and RNAses which may degrade samples.

Equipment needed:	**Reagents:**
10-15mL Wheaton homogenizers	RNAse free EtOH
rocker	sterile PBS
59ºC heat block (days 1 and 2)	RNAse OUT
50ºC heat block (day 3)	20mg/mL Proteinase K
vortex	TRIZOL Reagent (Invitrogen)
4ºC centrifuge (days 1 and 2)	phenol/chloroform reagent (Invitrogen)
4ºC microcentrifuge (days 2 and 3)	butanol
90ºC heat block (day 3)	isopropanol
	3M sodium acetate
	RNAse free water
	Buffer EB (Qiagen)

Recipes/Buffers:

1) Digestion Buffer: for 10mL
   500µL 1M Tris-HCl pH 8 (final=50mM)
   0.044g NaCl (final=75mM)
   50µL 1M $CaCl_2$ (final=5mM)
   0.01g SDS (final= 0.1%)
   ddH_2O to 10mL

2) Sodium citrate/ ethanol solution: for 10mL
   0.258g sodium citrate
   1mL 100% ethanol
   ddH_2O to 10mL

3) SNET Buffer: for 10mL
   0.1g SDS (final= 1%)
   0.234g NaCl (final=400mM)
   50µL 1M EDTA
   200µL Tris-HCl pH 8

DAY ONE: Tissue Processing

1) Deparaffinize tissue in 1mL of xylene @ room temp with rocking or shaking for 10min. Repeat twice.
2) Wash tissue in 1mL 100% EtOH @ room temp with rocking or shaking for 5min. Repeat twice.
3) Wash tissue in 1mL 95% EtOH @ room temp with rocking or shaking for 5min. Repeat twice.
4) Wash tissue in 1mL sterile 1X PBS for 1 min to rehydrate. Follow with incubation for 90min in 200µL sterile 1X PBS + 6.5µL RNAse OUT on ice.
5) Homogenize tissue in a clean Wheaton homogenizer using 2mL digestion buffer. Separate sample into five tubes containing 400µL each.
6) Add 2.8µL RNAse OUT to each tube. Add 60µL 20mg/mL Proteinase K to each tube. Incubate @ 59ºC for 1hr with vortexing every 5min.
7) Combine samples back into two 15mL tubes and spin down at 12000 rpm for 1 min.
8) Keep pellets on ice, add 1mL butanol to the supernatants. Vortex, centrifuge at 10000 rpm for 1 min. Pull off top layer and save as butanol phase (in case needed later). Repeat.
9) Resuspend tissue pellet in remaining lower phase. Should have around 150-200µL total volume.
10) Homogenize this solution in 1mL TRIZOL Reagent in a clean Wheaton homogenizer. Samples can be stored at this step @ room temp for several hours or at -80ºC for up to one month.
11) Incubate samples @ room temp for 5min.
12) Add 200µL phenol/chloroform to each tube. Shake or vortex vigorously for 15sec. Incubate 2-3 min @ room temp.
13) Incubate samples on ice for 10min to allow phase separation. Centrifuge at 12000xg for 15min @ 4ºC to further separate phases.
14) Remove the colorless upper aqueous phase and place into a new 15mL tube for RNA extraction. Save the interphase and lower red organic phenol/chloroform phase for DNA extraction. This can be stored at 4ºC overnight.

DAY TWO: RNA Isolation and Part one of DNA Isolation

<u>RNA Isolation:</u>

1) Add 500µL isopropanol to aqueous phase. Incubate @ room temp for 10min.
2) For further precipitation of RNA, add 1µL sodium acetate + 600µL ice-cold isopropanol. Incubate for 10min @ -20ºC. Centrifuge at 14000 rpm for 15min @ 4ºC.
3) Remove supernatant and leave RNA pellet on tube. Wash pellet with 1mL 75% EtOH. Vortex briefly, centrifuge @ 7500 xg for 5min @ 4ºC. Save supernatant as RNA wash (in case needed later)
4) Air dry pellet for 5-10min @ room temp. Do not allow pellet to dry completely.
5) Resuspend pellet in 50µL RNAse-free water by pipetting up and down. Incubate at 59ºC for 10-15min. Store at -80ºC.

DNA Isolation:

1) Remove any remaining aqueous phase in order to ensure higher quality DNA isolation.
2) Add 1.2mL 100% EtOH + 20µL 3M sodium acetate to the interphase/phenol/chloroform phases. Cap and invert several times to mix.
3) Incubate @ room temp for 3min, followed by centrifugation @ 16000 rpm for 20min @ 4ºC. Remove phenol-ethanol supernatant and store @ -80ºC for protein isolation or keep in case needed later.
4) Wash DNA pellet with 1mL sodium citrate/ethanol solution. Incubate for 30min @ room temp with occasional mixing by inversion. (Can stay in solution for up to 2 hours)
5) Centrifuge at 5000 xg for 5min @ 4ºC. Pull off supernatant and keep in case needed later.
6) Add 2mL 100% EtOH to wash pellet. Incubate 10min @ room temp with occasional inversion. Centrifuge at 5000 xg for 5min @ 4ºC. Pull off supernatant and keep in case needed later.
7) Air dry pellet at 50ºC for 5min. Do not allow pellet to dry out.
8) Add 400µL SNET buffer + 70µL 20mg/mL Proteinase K. Incubate at 56ºC overnight, with occasional vortexing before you leave.

***Overnight incubation is essential in order to remove protein that may have become cross-linked to DNA during formalin fixation. DNA cross-linked to protein will otherwise be lost in further washes.

DAY THREE: DNA Isolation

1) Incubate DNA sample @ 90ºC for 1hr in order to deactivate Proteinase K.
2) Precipitate DNA by adding 200µL ice-cold 75% EtOH. Incubate 10min @ room temp. Centrifuge @ 5000 xg for 10min @ 4ºC. Remove and discard supernatant. Repeat EtOH washes twice more.
3) Air dry pellet @ 50ºC for 5-10min. Add 100µL Buffer EB to resuspend pellet. Incubate @ 50ºC for 5-10min to further dissolve pellet. Pipette up and down to mix. Store at -80ºC.