ARGYLLA DNA nanoExtract Kit

Mini Scale, A11M011 (25-100 samples)
Standard Scale, A11M013 (125-500 samples)

ARGYLLA TECHNOLOGIES DNA & RNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY

FFPE Tissue Samples

This protocol describes how to isolate and purify Nucleic Acids, DNA and RNA, from Formalin-Fixed Paraffin-Embedded Tissue (FFPE) tissue samples.

It scales to elute to user determined Nucleic Acid concentrations. Additional concentration steps are typically not needed.

Resulting DNA eluates are ready for PCR analysis as long as the DNA preparation does not exceed 20% of the polymerase chain reaction volume. The protocol is flexible and readily scaled.

Additional protocols for this kit to extract DNA from the following sources can be accessed on our website:
♦Dried Blood Spots on Fibers ♦ Buccal Wash ♦ Samples Dried on Swabs ♦ Flash Frozen Tissue Thin Sections
Formalin-Fixed Paraffin-Embedded Tissue (FFPE) ♦ Cell-Free DNA from Serum (beta)

www.argylla.com/protocols

REAGENTS & CONSUMABLES

The Argylla DNA nanoExtract Kit includes:

PrepParticle Suspension	PN 100-00-00-S, 0.5mL PN 100-00-00-L, 2.5mL	Store in darkness & at room temp
20X Lithium Chloride Solution	PN 300-00-10-S, 1.25mL PN 300-00-10-L, 8.75mL	Caustic; eye, skin & respiratory irritant
20X Sarcosyl™ Solution	PN 310-00-30-S, 1.25mL PN 310-00-30-L, 6.25mL	Irritant to eye, skin, respiratory system
10X DNA Elution Buffer	PN 300-00-01-S, 0.5mL PN 300-00-01-L, 1.5mL	Irritant to eye, skin, respiratory system
20X DNA Extraction Buffer	PN 310-00-40-S, 1.5mL PN 310-00-40-L, 6.25mL	Irritant to eye, skin, respiratory system

Reagents to be supplied by user, as recommended by Argylla:

Savinase™ * ** Required but not included in the Argylla DNA nANOExtract Kit*	Argylla (PN 311-00-01-S), 25mL Argylla (PN 311-00-01-L), 100mL
8M Guanidine Hydrochloride (GuHCl)	Argylla (PN 310-00-20), 3.8 fl oz or Pierce (No. 24115); Fluka (No. 50937); Sigma-Aldrich (No. G9284)
Isopropanol, ACS-Grade	Sigma-Aldrich (No. I-9516)
Ethanol, methanol free, anhydrous Molecular Bio Grade (EtOH)	IBI Biochemicals (No. IB-15720)
Water, DNA-Grade	Fisher Scientific (No. BP2470-1)
5M Sodium Chloride (NaCl)	Sigma-Aldrich (No. S5150)
Costar Prelubricated Microfuge Tubes (silanized), 1.7mL	Costar (No. 3207)

Instructions for Isolating DNA and RNA from FFPE tissue sections

*** Preparation ***

- Set a heat-block to 95°C.

- It is important to thoroughly agitate the PrepParticle Suspension (PN 100-00-00) so that all aggregates are resuspended before each use.

Step 1. Prepare the following three solutions according to the number of samples to be processed.

Extraction Solution, ~200µL, (1per sample)

DNA Grade water	145µL
Argylla 20X Extraction Buffer (PN 310-00-40)	10µL
b-ME (*see footnote A)	5µL
8M GuHCl (*see footnote B)	35µL
Argylla 20X Sarcosyl (PN 310-00-30)	10µL

Ethanol Saline Rinse, 100mL, (0.5 to 1.5mL per sample)

Order of addition:		
	5M NaCl	dry Salt
1. NaCl: (use 5M NaCl or dry salt)	3.2mL	0.94 g
2. DNA Grade Water	21.8mL	25mL
3. 100% Ethanol (EtOH)	75mL	75mL

1X Elution Buffer Solution, 5mL, (for 50 samples)

DNA Grade Water	4.5mL
Argylla 10X Elution Buffer (PN 300-00-01) (*see footnote C)	0.5mL

*** Extraction ***

Step 2. Request that the FFPE sections be placed in 1.5 to 2mL polypropylene microfuge tubes. Dispense 200 µL Extraction Solution into a microfuge tube.

Heat at 95°C for 15 minutes.
Vortex periodically.

Step 3. Set the heat block to 56°C.

Step 4. Pulse centrifuge to separate paraffin from the Extraction Solution. A paraffin film may appear on the top. If so, puncture a hole in this film.

Step 5. When the temperature is at 56°C, add 5µL Savinase (PN 311-00-01). Vortex and Digest for 20 minutes or longer. (*see footnote B)

Step 6. Centrifuge at 10,000 xg for 5 minutes. The paraffin will be in a ring on the side of the tube. Transfer the supernatant to a new microfuge tube leaving the protein pellet in the old tube.

- If the digest is cloudy: Centrifuge the new tube without the paraffin for an additional 15 minutes at 10,000 xg. Transfer the supernatant to a new microfuge tube.

*** Purification ***

Step 7. *Before each use, thoroughly agitate the PrepParticle Suspension to an even suspension, free of aggregates.*

Add 10µL of PrepParticle Suspension (PN 100-00-00).
Add 11µL 20X Lithium Chloride Solution (PN 300-00-10).
Mix by vortex.

Step 8. Add 480µL Isopropanol, mix by vortex.
Let sit at room temperature for 5-10 minutes.

Step 9. Centrifuge for 2 minutes at 2000 xg, then carefully withdraw and discard the supernatant. **Retain the pellet.**

Step 10. Rinse the pellet 1-3 times with 0.5 mL Ethanol Saline Rinse solution.

Add 500µL Ethanol Saline Rinse and gently pipette up and down to disperse and rinse the pellet. Centrifuge the sample for 2 minutes at 2000 xg and then carefully remove as much supernatant as possible by pipet**. **Retain the pellet.**

- If protein carryover is a concern, monitor the absorbance of the supernatant at 280 nm and repeat if desired.

***It is imperative that as much alcohol-containing wash solution be withdrawn from the pellet as possible at this point. We recommend withdrawing visible rinse solution with a P-20 tip. Unnecessary alcohol carryover may inhibit DNA amplification downstream.*

Step 11. Allow residual alcohol to evaporate from uncapped tubes. (small hairline cracks appearing in pellet is a good indicator that the pellet is dry)

*** **Elution and Concentration** ***

Step 12. Elute DNA from the pellet with 100 µL 1X Elution Buffer Solution. Allow the pellet to rehydrate for 5-15 minutes at 56°C. Then gently pipette or vortex to fully disrupt and resuspend the pellet to a slurry. No particle aggregates should be visible in suspension.

- TE, or other desired solution may be substituted for Argylla Elution Buffer (PN 300-00-01)
- Volume of Elution Buffer may be adjusted up or down for desired concentration.

Step 13. Centrifuge the suspension for 5 minutes at 8000 xg to precipitate the spent PrepParticles from the DNA-containing solution. **Retain the supernatant**, which is the final DNA-containing eluate, and transfer to a new tube.

***Footnotes:**

A) Alternatives to b-ME include 100mM DTT (similar mercaptan odor) from a 1M stock and TCEP (odorless) from a 0.5M stock. Argylla has obtained best results from b-ME.

B) One may also use 10µL GuHCl and 165µL water (400mM GuHCl) and an overnight digestion if subsequent use of DNAse or RNAse is desired that might be sensitive to 1.4M GuHCl. (not tested by Argylla)

C) If using Argylla Elution Buffer (PN 300-00-01), sample volume should be less than 10% of total PCR reaction volume. Elution Buffer contains: 0.1 mM EDTA, 0.001% Tween 20, and 10mM Sodium Tetraborate. Current testing suggests any buffer in the pH 7.5-9 will work well. The optimal elution volume will have to be determined by the user. We suggest starting at 100 µL per 40 µm section.

- Please note that the use of high quality silanized plastic labware, such as pipette tips and tubes, is essential to the maximum recovery of small amounts of DNA. These surfaces can represent the majority of systematic losses when processing small samples. With proper handling and quality labware the Argylla DNA nanoExtract kit can process and deliver as little as 100 picograms of DNA.