ARGYLLA DNA NanoExtract Kit

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

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY,

Thin Section Tissue Samples

This protocol describes how to isolate and purify Nucleic Acids, DNA and RNA, from frozen thin section 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
8M Guanidine Hydrochloride	PN 310 00 20-3.6 mL PN 310 00 20-18 mL	May be hazardous – see MSDS www.argylla.com/downloads.html

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 or Sigma-Aldrich No. P3111
8M Guanidine Hydrochloride	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	IBI Biochemicals No. IB-15720
Water, DNA-Grade	Fisher Scientific No. BP2470-1
5M Sodium Chloride (NaCl)	Sigma-Aldrich No. S5150
1M Tris-Hcl, pH 8.0	Gibco-BRL No. 15568-025
Costar Prelubricated Microfuge Tubes (silanized), 1.7mL	Costar no. 3207

The following are registered trademarks: Sarcosyl (Ciba-Geigy), Savinase (Novozyme Corp.), Sigma-Aldrich, Fluka, Pierce, Argylla Technologies, PrepParticle(s) and the phrases PrepParticle NanoChromatography and Colloidal NanoChromatography (Argylla Technologies, LLC)

Instructions for Isolating DNA and RNA from Thin Section tissue sections.

*** Preparation ***

- Set a heat-block to 56^{0}C

- It is important to thoroughly agitate the PrepParticle Suspension 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. If you are using frozen tissue slices taken directly from the cryostat, chill forceps and the 1.5mL polypropylene tubes on dry ice. Wearing thick gloves or wrapping the forceps in a paper towel slows the transfer of heat from your hand to the forceps.

Step 3. Dispense 100 µL of the Extraction Solution into each tube. Rinse the sides of the tubes by pipetting extraction solution over the frozen tissue that has melted on the sides of the tubes. If some of the thin section melted on the forceps while being transferred to the tube, rinse the melted tissue on the forceps into the tube with the 100µL Extraction Buffer in the tube.

Step 4. Place the tubes in the heat block. Wait 5 minutes to come to temperature. Vortex. Note the appearance. The goal is to have a largely clear solution with small amounts of fibrous material. Keep adding Extraction Buffer in 100µL aliquots followed by pipetting up and down. Allow 5 min between additions. Stop when "Step 4" at the top of this paragraph can be read when the tube is held up against these printed instructions.

Step 5. When the tissue is largely dissolved, add 5µL Savinase for every 100µL of sample. Vortex and Digest for 20 minutes or longer. (see footnote B)

Step 6. Centrifuge at 10,000xg for 5 minutes. Retain the supernatant and transfer to a polypropylene tube capable of holding 3.2x the volume of the supernatant.

*** Purification ***

Step 7. *Thoroughly agitate the PrepParticle Suspension to an even suspension, free of aggregates, before each use.*
Add 5µL PrepParticles (PN 100 00 00) and 5.5 µL LiCl (PN 300 00 10) for every 100µL of the digest. Vortex.

Step 8. Add two times the volume of the supernatant in 100% 2PrOH. Mix by vortexing. Let sit at room temperature for 5-10 min.

Step 9. Centrifuge for 2min at 2000 x g. There should be a firmly packed pellet with occasionally what appears to be a looser packed lipid layer. Carefully withdraw and discard as much of the supernatant as possible. **Retain the pellet.**

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

Add 500µL Ethanol-Saline Rinse Solution and gently pipette up and down to disperse and rinse the pellet. Centrifuge the sample for 2min at 2000 x g 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. Be aware that all chromatography particles can break up with handling. Be gentle and do not handle more than necessary.

➢ It is imperative that as much alcohol-containing wash solution be withdrawn from the pellet as possible at this point. We recommend withdrawing visible wash 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 Argylla Elution Buffer. Allow the pellet to rehydrate for 5-15min 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. Large amounts of genomic sized DNA may require more time.

- TE, or other desired solution may be substituted for Argylla Elution Buffer. (See footnote C)
- Volume of elution buffer may be adjusted up or down for desired concentration.

Step 13. Centrifuge the suspension for 5min at 8,000 x g 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 GnHCl and 165µL water (400mM GnHCl) and an overnight digestion if subsequent use of DNAse or RNAse is desired that might be sensitive to 1.4nM GnHCl. (not tested by Argylla)

C) If using Argylla Elution buffer, sample volume should be less than 10% of total PCR reaction volume. 1X 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 µL 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.