

# ARGYLLA DNA nanoPurify Kit

A10M011 (25-100 samples)

A10M013 (125-500 samples)

## ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY, FORMALIN-FIXED PARAFFIN-EMBEDDED (FFPE) TISSUES

The following protocol outlines the extraction and purification of DNA from sectioned formalin-fixed paraffin-embedded (FFPE) tissues. It has been optimized for sections measuring 50µm. Depending on the samples' tissue to paraffin mass ratio, the anatomical source and relative disease state of the sample, DNA yields ranging from 1ng/µL to 200ng/µL may result. Common observations fall in the 10ng/µL to 99ng/µL range. FFPE samples archived only briefly or for many years will yield DNA using this protocol and resultant DNA eluates are ready for PCR analysis as long as the DNA preparation does not exceed 20% of the total reaction volume. Additional concentration steps are typically not needed. This is an ideal process for small or dilute samples with as little as 100 picograms DNA content as systematic losses are minimal.

Additional application protocols can be accessed on our website:

[www.Argylla.com/downloads](http://www.Argylla.com/downloads)

Whole Blood or Buffy Coat ♦ Dried Blood Spots on Paper ♦ Samples Dried on Swabs ♦ Buccal Wash  
Flash Frozen Tissue Thin Sections ♦ Formalin-Fixed Paraffin-Embedded Tissue (FFPE)  
Cell-Free DNA from Serum (beta)

### REAGENTS & CONSUMABLES

#### The Argylla DNA nanoPurify 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

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

Proteinase K Enzyme at 20mg/mL or >600mAU/mL	Qiagen No. 19131, 19133
Qiagen Buffer ATL Tissue Lysis Buffer	Qiagen No. 19076
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), Qiagen Buffer ATL Tissue Lysis Buffer (Qiagen), Costar (Corning), Sigma-Aldrich, Fluka, Pierce, Argylla Technologies, PrepParticle(s) and the phrases PrepParticle NanoChromatography and Colloidal NanoChromatography (Argylla Technologies, LLC)

- Please note that the use of high quality silanized plastic labware, such as pipet 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 NanoPurify Kit can process and deliver as little as 100 picograms of DNA.

**Instructions for Isolating DNA from Formalin-Fixed Paraffin-Embedded Tissues**

**\*\*\* Dissolution \*\*\***

The following must be performed in 1.7mL prelubricated (*i.e.*, silanized) round-bottom microfuge tubes; it may be necessary to carefully transfer archived sections of tissue-containing paraffin from their storage vessels using small forceps (Step 3). Immersing forceps in isopropanol and drying with delicate-task wipes between transfer of different samples will prevent sample cross-contamination.

**Day 1.**

1. Set a heat-block at **98°C**
2. Add 100µL Qiagen Buffer ATL Tissue Lysis Buffer to round-bottom 1.7mL microfuge tubes and pulse-centrifuge to partition buffer to the bottom of the tube.
3. Add microdissected section to the pooled Buffer and repeat pulse-centrifugation, immersing much of the section in Lysis Buffer.
4. Place the tubes on a heat block at 98°C for 15min. Remove tubes from the block and change the block's temperature setting to 56°C.
5. Cool samples at room temperature for 15min and reset the heat block to 56°C.
6. Perform a pulse-centrifugation to pellet condensation then add 12µL Proteinase K (20mg/mL or >600 mAU/ml, solution). Vortex and pulse-spin the preparation.
7. Return the samples to a 56°C heat block (+/- 2°C) overnight.  
 ✓ If the heat block has not yet cooled to this temperature, Proteinase K may be denatured and the DNA extraction procedure will be compromised.

**Day 2.**

**MORNING:**

8. A wax overlay will harden atop each digest overnight. Pierce a hole in this "cap" with a sterile P20 tip. A small wax plug will enter that tip so discard it. Using a new sterile pipet tip, enter the solution through the perforation and expel 12µL Proteinase K directly into the fluid volume beneath the wax cap. Perform a pulse spin to repellet condensate.
9. Incubate for at least 5hrs at 56°C.

**EVENING**

10. Repeat Step 8.
11. Return samples to incubation at 56°C overnight.

**Day 3. Extraction, Purification, and Concentration**

**\*\*\* Preparation \*\*\***

- Set a heat-block to 56°C
  - It is important to thoroughly agitate the PrepParticle Suspension so that all aggregates are resuspended before each use.
12. Prepare the following two solutions according to the number of samples to be processed. Each solution's components should be added in the order listed.

**Ethanol-Saline Wash Solution** : store refrigerated and use within 7 days

Component	µL per sample	x Number of Samples	= Total Vol	<b>Example:</b> 15 samples
Water	333µL			15 x 333µL = 5mL
Ethanol	667µL			15 x 667µL = 10mL
5M NaCl Solution	30µL			15 x 30µL = 450µL
				15.5mL Ethanol-Saline Wash

**1X DNA Elution Buffer:** use on date prepared

Component	µL per sample	x Number of Samples	<b>Example: 15 samples</b>
Water	45		45µL x 15 = 675µL
10X DNA Elution Buffer (PN 300 00 01)	5		5µL x 15 samples = 75µL
			750µL 1X DNA Extraction Buffer

**\*\*\* Extraction and Purification \*\*\***

13. Pulse spin samples then pierce the wax cap with a sterile P20 pipet tip. Using a new tip, enter the solution through this perforation and withdraw all liquid from beneath the wax. Transfer the viscous liquid to a new 1.7mL tube. Do not use the same tip to pierce the wax and to withdraw the liquid as this will result in the transfer of wax to into the DNA isolation steps.
14. Raise the volume in each tube to 500uL with molecular biology grade water (anticipated to require 315 - 345µL water). Sequentially:
  - a. Add 25µL 20X Sarcosyl then vortex.
  - b. Add 10µL Argylla PrepParticle Suspension and vortex again.
  - c. Add 25µL 20X Lithium Chloride Solution and vortex briefly.
  - d. Slowly add 1mL (1000µL) isopropanol and vortex; 1.7mL tube will be nearly full.
15. Allow the tube to incubate at room temperature for 30min.
16. Perform centrifugation at 4000 x *g* for 2min.
  - ✓ User may consult the rotor speed conversion chart at the end of this protocol if their instrument does not automatically convert rotations per minute (rpms) to relative centrifugal forces (rcf's), described in *g*-forces.
17. Discard the supernatant. **Retain the pellet.** Add 1mL of "Etahnol-Saline Wash Solution" as prepared in Step 12 and vortex briefly, rinsing both the surface of the vessel and the pellet. Pellet may or may not disperse in this wash step.
18. Spin again at 4000 x *g* for 2min. Discard the supernatant and retain the pellet. Spin at 4000 x *g* for 30sec and carefully withdraw remaining Ethanol-Saline Wash Solution from the pellet. **Retain the pellet.**
  - ✓ *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.*
19. Allow residual alcohol to evaporate from the pellet in its uncapped microfuge tube for 10min.

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

20. Add 50µL "1X Argylla Elution Buffer". Recap the tube but do not mix/agitate at this point. Place the tube in a 56°C heat block for 10min.
  - ✓ Elution volume may vary from 5 – 100µL. The ratio of tissue to paraffin content in the initial sample can only be estimated by the User. However, 50µL 1X DNA Elution Buffer volume is recommended as a general guideline.
21. After 15min of rehydration, vortex the pellet, resuspending to a slurry and return samples to 56°C heat block. Repeat the vortex/15min 56°C incubation cycle 2 - 3 times, until particle aggregates are no longer visible in suspension.
  - ✓ The greater the DNA content of your sample (*i.e.*, the greater the input tissue mass in Step 3) the more vortex/56°C incubation cycles will be required to resuspend the pellet since DNA is a cohesive species, apt to slow dispersion of the colloidal nanoparticles. A large FFPE sample may require up to 5 vortex/56°C incubation cycles for resuspension and optimal DNA yield.

Once resuspended, further incubate the PrepParticle slurry at 56°C for 30min. This will ensure maximal DNA recovery.
22. Centrifuge the suspension for 5min at 8,000 x *g* to precipitate the spent PrepParticles from the DNA-containing solution.
23. **Retain the supernatant**, which is the final DNA-containing eluate, and transfer to a new tube.
  - ✓ If PrepParticles are transferred with DNA eluate, repeat Step 22 for 10min and transfer the clarified supernatant to a new tube

The DNA recovered is highly pure and suitable for DNA-based molecular biological studies.

**\*\*See Storage Note\*\***

**\*\*\* Storage Note \*\*\***

DNA eluates are stable for long term storage at 4°C. 1M Tris - HCl, pH 8.0, may be added to the eluate to alter its pH to ~8.2 at 1/20<sup>th</sup> the 1X Elution Buffer volume (0.5µL per 10µL eluate) if DNA storage at -20°C or -80°C is planned. This final DNA eluate, with or without Tris-HCl, is suitable for PCR as long as the DNA supernatant does not exceed 20% of the total PCR reaction volume.

**"G" Force (RCF) Determination Based On RPM and Rotor Radius.**

RPM	Rotor Radius in centimeters											
	4	5	6	7	8	9	10	11	12	13	14	15
3,500	548	685	822	959	1096	1233	1370	1507	1643	1780	1917	2054
4,000	716	894	1073	1252	1431	1610	1789	1968	2147	2325	2504	2683
4,500	906	1132	1358	1585	1811	2038	2264	2490	2717	2943	3170	3396
5,000	1118	1398	1677	1957	2236	2516	2795	3075	3354	3634	3913	4193
5,500	1353	1691	2029	2367	2706	3044	3382	3720	4058	4397	4735	5073
6,000	1610	2012	2415	2817	3220	3622	4025	4427	4830	5232	5635	6037
6,500	1889	2362	2834	3306	3779	4251	4724	5196	5668	6141	6613	7085
7,000	2191	2739	3287	3835	4383	4930	5478	6026	6574	7122	7669	8217
7,500	2516	3144	3773	4402	5031	5660	6289	6918	7547	8175	8804	9433
8,000	2862	3578	4293	5009	5724	6440	7155	7871	8586	9302	10017	10733
8,500	3231	4039	4847	5654	6462	7270	8078	8885	9693	10501	11309	12116
9,000	3622	4528	5433	6339	7245	8150	9056	9961	10867	11773	12678	13584
9,500	4036	5045	6054	7063	8072	9081	10090	11099	12108	13117	14126	15135
10,000	4472	5590	6708	7826	8944	10062	11180	12298	13416	14534	15652	16770
10,500	4930	6163	7396	8628	9861	11093	12326	13559	14791	16024	17256	18489
11,000	5411	6764	8117	9469	10822	12175	13528	14881	16233	17586	18939	20292

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