ARGYLLA DNA NanoPurify Kit

A10M011 (25-100 samples)
A10M013 (125-500 samples)

ARGYLLA TECHNOLOGIES PROTOCOL FOR FOR PURIFYING AND CONCENTRATING DNA

The Argylla DNA NanoPurify Kit, based on Nanochromatography, is for purifying and further concentrating DNA from samples with as little as 100 picograms up to 15 micrograms of DNA. This approach avoids the need for carrier DNA, RNA, glycogen or other coprecipitants that might compromise downstream molecular biology applications. The primary applications of this kit are:

1.) To process DNA from samples lysed by other front end proceses.
2.) To process DNA from reactions such as PCR, whole genome amplification (WGA), enzyme digests or labeling reactions,
3.) To further purify and concentrate DNA extracted by methods based on spin columns, magnetic beads, microfiltration, protease-digests or organic solvent extraction,
4.) To further concentrate dilute DNA eluates isolated at dilute concentrations by any of these means.

The process ensures very high input to recovery ratios and eluted DNA demonstrates the integrity to support enzyme labeling, PCR, WGA, Southern blotting and other hybridization assays.

Additional application protocols can be accessed on our website:
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:

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
Tris-EDTA Buffer (10mM Tris-HCl/1mM EDTA, pH 8)	Fluka no. 93283
1M Tris-Hcl, pH 8.0	Gibco-BRL No. 15568-025
Costar Prelubricated Microfuge Tubes (silanized), 1.7mL	Costar no. 3207

- 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 Arylla DNA NanoPurify Kit can process and deliver as little as 100 picograms of DNA.

ARGYLLA TECHNOLOGIES PROTOCOL FOR PURIFYING AND CONCENTRATING DNA

Instructions for **Purifying and Concentrating DNA**

***** 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.

Step 1. 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.

75% Ethanol-Saline Wash Solution: store refrigerated and use within 7 days

Component	µL **per sample**	**X Number of Samples**	**= Total volume**	**Example: 25 samples**
Water	107.5µL	X	=	25 x 107.5µL = 2.69mL
Ethanol	376µL	X	=	25 x 375µL = 9.4mL
5M NaCl Solution	16.1µL	X	=	25 x 16.1µL = 402.5µL
				12.5mL Ethanol-Saline Wash

1X DNA Elution Buffer

Component	**Eluting In 10µL**	**Eluting In 50µL**	**Eluting in 100µL**	**Eluting in 150µL**	**X Number of Samples**	**Example:** 25 samples eluted in 50uL Elution Buffer
Water	9µL	45µL	90µL	135µL		45uL x 25 = 1125µL
10X DNA Elution Buffer (PN 300 00 01)	1µL	5µL	10µL	15µL		5µL x 25 = 1 25µL
						1.25mL 1X DNA Extraction Buffer

***** Capture and Purification *****

Step 2. Place the DNA sample (pH 6.0 – 10.5) in a standard silanized microfuge tube and adjust the sample's volume to 500µL (+/- 25µL) with standard Tris-EDTA Buffer Solution, pH 8.0.

✓ Beware not to confuse Tris-EDTA with 1M Tris-HCl, called for elsewhere in this protocol.

Step 3. Add 25µL of 20X Sarcosyl Solution and mix preparation by vortexing.

✓ N-lauroylsarcosine serves as a blocking agent that prevents DNA from adsorbing to non-specific surfaces, inhibits nucleases and proteins from binding to the target species, and reduces the carry-over of SDS into the DNA eluate thus purified. A 20% solution may be prepared from the molecular biology grade solid, if the user prefers.

Step 4. *Thoroughly agitate the PrepParticle Suspension to an even suspension, free of aggregates, before each use.*

Add 10µL PrepParticle Suspension (PN 100 00 00) and **mix thoroughly** by vortexing.

Step 5. Add 25µL 20X Lithium Chloride Solution (PN 300 00 10).
Mix and then incubate at 56°C for 5min to dissolve flocculated material that may have formed in the extraction solution.

Step 6. Add 575µL isopropanol, mix by vortexing and incubate for 10 min.
Repeat mixing and incubate for an additional 20 – 30 min.

Step 7. Centrifuge at 4000 x *g* for 5min to pellet the suspended DNA-bound PrepParticles. Remove the supernatant and discard. **Retain the pellet.**

✓ User may consult the rotor speed conversion chart (nomogram) at the end of this protocol if their centrifuge does not automatically convert rpm to relative centrifugal forces (g's) .

Step 8. Add 500µL Ethanol-Saline Wash Solution and mix vigorously by vortex to rinse the pellet and tube's surface. The pellet *may* be dislodged from the sidewall. Centrifuge at 4000 x *g* for 2min to pellet any resuspended DNA-coated PrepParticles and ensure optimal yield. Remove the supernatant by pipet** and discard. **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.*

Step 9. Allow residual alcohol to evaporate from uncapped tubes for 10min at room temperature.

*** Elution and Concentration ***

Step 10. To elute DNA from the PrepParticles, add from10 up to 150µL of 1X DNA Elution Buffer directly to the surface of the pellet. *Do not agitate the tube's contents at this point* but allow 1X DNA Elution Buffer to rehydrate the pellet for 15min at 56°C.

Step 11. After 15min rehydration, vortex rehydrated pellet to resuspend to a slurry and return samples to 56^{0}C heat block for another 15min. Repeat the vortex and 15 min 56°C incubation cycle 1-3 times, until particle aggregates are no longer visible in suspension.

- ✓ The greater the DNA content of your sample (up to15µg) the more heating and agitation cycles will be required to resuspend the pellet since DNA is a very cohesive species.

Once resuspended, further incubate the PrepParticle slurry at 56^{0}c for 30min. This will ensure maximal DNA recovery.

Step 12. Centrifuge the suspension for 10min at 8000 x *g* to precipitate the spent PrepParticles from the DNA-containing solution.

Step 13. Retain the supernatant, which is the final DNA-containing eluate.

- ✓ If PrepParticles are transferred with DNA eluate, repeat Step 10 for 5min 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^{0}C. 1M Tris - HCl, pH 8.0, may be added to the eluate to alter its pH to ~8.2 at 1/20th the 1X Elution Buffer volume (0.5µL per 10µL eluate) if DNA storage at -20^{0}C or -80^{0}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.

	Rotor Radius in centimeters											
RPM	**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	16223	17586	18939	20292

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