ARGYLLA DNA nanoXtract Kit

A11M011 (25-100 samples)
A11M013 (125-500 samples)

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY,
DRIED BLOOD STORED ON PAPER

The following Argylla protocol describes how to purify DNA from dried blood spots formed on Guthrie cards, FTA paper or other filter substrates. It is assumed that the sample consists of small paper discs punched out from a larger card, these discs or "chads" measuring from 2mm to 10mm in diameter. As a point of reference, a typical 6mm blood-permeated chad may contain between 0.2µg – 0.4µg of DNA.

It elutes to user determined DNA 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. 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
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 nanoXtract 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- 3.8 PN 310 00 20-18	May be Hazardous; see MSDS

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

Savinase™	Argylla PN 311 00 01-S, 25mL Argylla PN 311 00 01-L, 100mL or Sigma-Aldrich No. P3111
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

- 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 NanoExtract kit can process and deliver as little as 100 picograms of DNA.

Instructions for Isolating DNA from Dried Blood Stored on Paper

*** **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. Each solution's components should be added in the order listed.

2X DNA Extraction Buffer: store at room temperature and use on date prepared

Component	µL per sample	X Number of Samples	= volume	Example: 25 samples
Water	250µL	X	=	25 x 250µL = 6.25mL
20X DNA Extraction Buffer (PN 310 00 40)	50µL	X	=	25 x 50 = 1.25mL
20X Sarcosyl Solution (PN 310 00 30)	50µL	X	=	25 x 50 = 1.25mL
8M Guanidine Hydrochloride (PN 310 00 20)	150µL	X	=	25 x 150 = 3.75mL
				12.5mL 2X DNA Extraction Buffer

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	250µL	X	=	25 x 250µL = 6250µL
Ethanol	250µL	X	=	25 x 250µL = 6250µL
5M NaCl Solution	15µL	X	=	25 x 15µL = 375µL
				Ethanol-Saline Wash 12.88mL

1X DNA Elution Buffer: use on date of preparation.

Component	Eluting In 10µL:	Eluting in 20µL:	Eluting In 35µL:	Eluting In 50µL:	Eluting in 75µL:	Eluting in 100µL:	Example: 25 samples eluted in 50µL Elution Buffer
Water	9µL	18µL	31.5µL	45µL	67.5µL	90µL	45µL x 25 samples =1.125mL
10X DNA Elution Buffer (PN 300 00 01)	1µL	2µL	3.5µL	5µL	7.5µL	10µL	5µL x 25 samples = 125uL
							1.25mL 1X DNA Extraction Buffer

*** **Extraction** ***

Step 2. Prepare the following Extraction Master Mix immediately prior to the DNA isolation procedure and mix well by inversion:

Component	Volume	X Number of Samples	= Vol. of Component	Example: 10 individual swabs
2X DNA Extraction Buffer (from Step 1)	250µL			10 x 250µL = 2.5mL
Water, molecular biology grade	225µL			10 x 225µL = 2.25mL
Savinase™	75µL			10 x 75µL = 750µL
				5.5mL Master Mix

Step 3. Place blood spotted chad into a microfuge tube and immerse the chad in the solution. Incubate at 56^{0}C for at least 4 hours to overnight, occasionally agitating the sample by vortexing briefly.

- ✓ Overnight digestion is especially useful if the blood sample has been archived for longer than 1 month.

Step 4. Perform a pulse centrifugation step to precipitate paper, then transfer the supernatant to a new microfuge tube with a pipettor. Discard the spent paper.

Step 5. Centrifuge for 10min at 10,000 x g to pellet remaining suspended solids. Transfer the clarified supernatant to a new 1.7mL silanized microfuge tube.

- ✓ If the supernatant appears clouded, repeat centrifugation and transfer of this supernatant to a new tube.
- ✓ User may consult the rotor speed conversion chart at the end of this protocol if their centrifuge does not automatically convert rotations per minute (rpm) to relative centrifugal forces (g's).

***** Purification *****

Step 6. *Thoroughly agitate the PrepParticle Suspension to an even suspension, free of aggregates, before each use.*

Add 5uL to 10µL PrepParticle Suspension and mix thoroughly by vortexing.

- ❖ Utilize 10µL PrepParticle Suspension for 6mm – 10mm paper punches.
- ❖ 5µL PrepParticle Suspension may be used for 2mm – 4mm chads.
- ✓ A 5µL pellet will be quite small and care must be exercised when pipetting in Step 9 to avoid discarding it. Use of up to 10µL PrepParticle Suspension eases pellet visualization.

Step 7. 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 8. Add 550µL isopropanol, mix by vortex, and then allow sample to incubate at room temperature for 10min. Vortex briefly, then continue incubating for another 20 - 30min.

Step 9. Centrifuge for 5min at 4000 x g then carefully withdraw and discard the supernatant. **Retain the pellet**.

Add 500µL Ethanol-Saline Wash Solution and vortex tube to wash the pellet. Pellet may become dislodged from the tube's wall.

Centrifuge the sample for 2min at 4000 x g and then carefully remove as much supernatant as possible by pipet**. **Retain the pellet**. DNA is bound to 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 10. Allow residual alcohol to evaporate from uncapped tubes for 10min.

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

Step 11. To elute DNA from the PrepParticles, add 10 to 50µL of 1X DNA Elution Buffer directly to the surface of the pellet. *Do not agitate the tube's contents* but allow 1X DNA Elution Buffer to rehydrate the pellet for 15min at 56°C

Step 12. After 15min rehydration, vortex the rehydrated pellet to resuspend to a slurry and return samples to 56°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 *(i.e*, the greater the number of nucleated cells input~~ted~~ in Step 2.) 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°c for 30min. This will ensure maximal DNA recovery.

Step 13. Centrifuge the suspension for 5min at 8,000 x g to precipitate the spent PrepParticles from the DNA-containing solution.

Step 14. 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 13 for 10min.

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

See Storage Notes

***** Storage Notes *****

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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