

ARGYLLA DNA nanoXtract Kit

A11M011 (25-100 samples)

A11M013 (125-500 samples)

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY, SAMPLES DRIED ON SWABS

The following protocol describes the extraction and purification of DNA from blood, semen, buccal cells or skin samples collected on swabs using NanoChromatography. Many factors influence the yield of DNA purified from a swab, including the number of nucleated cells collected, immediate sample handling, shipping & storage conditions and fixation/long-term preservation measures. For example, a typical buccal swab may contain from 0.2µg to 50µg of DNA (see references 1 & 2) while an epithelial swab, as collected from the skin's surface, may contain only 1ng – 100ng of DNA. Depending on these factors, a DNA eluate obtained using this PrepParticle NanoChromatography protocol can be retrieved at a yield of 0.1µg up to about 5µg pure material. The process elutes to user determined DNA concentrations in the range of 1-10ng/µL. Additional concentration steps are not typically 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.

- 1.) Freeman, B. *et al.* (2003) DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. *Behav Genet*; 33:67-72.
- 2.) Zheng, S. *et al.* (2001) Whole genome amplification increases the efficiency and validity of buccal cell genotyping in pediatric populations. *Cancer Epidemiol Biomarkers Prev*; 10:697-700.

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
8M Guanidine Hydrochloride	PN 310 00 20 - 3.8 PN 310 00 20 - 18	May be harmful; see MSDS
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™	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

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- Please note that use of high quality silanized plasticware, such as pipette tips & tubes, is essential to the maximum recovery of small amounts of DNA. Residual, untransferred volume lost to 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.

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Instructions for Isolating DNA from Samples Dried on Swabs

***** 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 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: use on date prepared

Component	µL per sample	X Number of Samples	= Volume	Example: 25 samples
Water	150µL	X	=	25 x 150µL = 3.75mL
20X DNA Extraction Buffer (PN 310 00 40)	30µL	X	=	25 x 30µL = 750µL
20X Sarcosyl Solution (PN 310 00 30)	30µL	X	=	25 x 30µL = 750µL
8M Guanidine Hydrochloride (PN 310 00 20)	90µL	X	=	25 x 90µL = 2.25mL
				7.5mL 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
				12.88mL Ethanol-Saline Wash

1X DNA Elution Buffer: use on date prepared

Component	Eluting in 50µL: Vol./sample	Eluting in 100µL: Vol./sample	Example: 25 samples eluted in 50µL Elution Buffer
Water	9µL	45µL	9µL x 25 = 225 µL
10X DNA Elution Buffer (PN 300 00 01)	1µL	5µL	5µL x 25 samples = 125µL
			350µL 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	µL	X Number of Samples	= Vol. of Component	Example: 10 individual swabs
2X DNA Extraction Buffer (from Step 1)	300µL			10 x 300µL = 3mL
Water	200µL			10 x 200µL = 2mL
Savinase™	100µL			10 x 100µL = 1mL
				6mL Master Mix

Step 3. Place each swab into a 1.7mL silanized microfuge tube.

- ✓ Tubes that include spin baskets for gross filtration may be used in this step.

Add 600µL Extraction Master Mix (Step 2) and immerse the swab in the solution. Incubate at 56°C for 4 hours to overnight, occasionally agitating the sample by vortexing briefly.

- ✓ Overnight digestion is especially useful if the swab was air-dried for many days or was alcohol-fixed for storage or shipment.

Step 4. Transfer liquid material (*i.e.*, material other than the swab) to a new microfuge tube with a pipettor. Discard the spent swab.

Step 5. Centrifuge for 10min at 10,000 x g to pellet remaining suspended solids.

Thoroughly transfer the clarified supernatant to a new 1.7mL silanized microfuge tube.

- ✓ User may consult the rotor speed conversion chart at the end of this protocol if their centrifuge does not automatically convert rotations per minuter (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 PrepParticle Suspension (PN 100 00 00) and **mix thoroughly** by vortexing.

- ❖ Buccal cell swabs or swabs from large blood volumes are best processed using 10µL PrepParticle Suspension, as these swabs will hold an abundance of cells.
- ❖ Other surface swabs may use 5µL PrepParticle Suspension.
- ✓ 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. Carefully add 650µL isopropanol, mix by vortexing and incubate for 10 min. Repeat mixing and incubate for an additional 20 – 30 min.

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 “1X DNA Elution Buffer” directly to the surface of the pellet. (Volume of buffer can be adjusted for desired concentration)

- ❖ A buccal swab or swab containing more than 10µL blood volume is preferably eluted in 50µL of 1X DNA Elution Buffer.
- ❖ A surface or skin swab is not expected to hold abundant DNA material and is preferably eluted in 10µL of 1X DNA Elution Buffer.

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 allowing 15min for 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 processed 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 Note

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***** 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 1/20th 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.

	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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