

# ARGYLLA DNA nanoXtract Kit

A11M011 (25-100 samples)

A11M013 (125-500 samples)

## ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY, WHOLE BLOOD & BUFFY COAT 0.05µL – 200µL

This protocol describes how to isolate and purify DNA from blood samples, including fresh peripheral blood (PBL), buffy coat or enriched lymphocyte fractions from a Ficoll gradient or from erythrocyte depletion via saponin-mediated lysis.

This protocol scales in sample size from 0.05µl to 200µL, with minor yet consequential variations for samples less than 50µL. Depending on initial sample size, the PrepParticle protocol will yield about 1µg of pure DNA per 50µL of whole blood starting material. It also scales to elute 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. The protocol is flexible and readily scaled volumetrically, up to 8mL (contact ARGYLLA TECHNOLOGIES for details 1-800-234-6642). This is an ideal process for small or dilute samples with as little as 100 picograms DNA content as systematic losses are minimal.

Application Protocols to extract DNA from the following sources can be accessed on our website:

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

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 00-0.5 mL<br>PN 100 00 00-2.5 mL | Store in darkness & at room temp          |
| 20X Lithium Chloride Solution | PN 300 00 10-1.25mL<br>PN 300 00 10-8.75mL | Caustic; eye, skin & respiratory irritant |
| 20X Sarcosyl™ Solution        | PN 310 00 30-1.25mL<br>PN 310 00 30-6.25mL | Irritant to eye, skin, respiratory system |
| 10X DNA Elution Buffer        | PN 300 00 01-0.5mL<br>PN 300 00 01-1.5mL   | Irritant to eye, skin, respiratory system |
| 20X DNA Extraction Buffer     | PN 310 00 40-1.5mL<br>PN 310 00 40-6.25mL  | Irritant to eye, skin, respiratory system |
| 8M Guanidine Hydrochloride    | PN 310 00 20-3.6 mL<br>PN 310 00 20-18 mL  | May be Hazardous – see MSDS               |

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

|  |   |
|--|---|
| *Savinase™<br>* Required but not included in the ARGYLLA DNA NANOExtract Kit | Argylla PN 311 00 01-S, 25mL<br>Argylla PN 311 00 01-L, 100mL or<br>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 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.

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY, WHOLE BLOOD & BUFFY COAT 0.05µL – 200µL

❖ Indicates different volumetric conditions, depending on initial volume of sample

**Instructions for Isolating DNA from 0.05µL – 200µL Whole Blood or Buffy Coat**

\*\*\* 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 <50µL sample     | µL per 50-200µL sample | X Number of Samples | = volume | Example: 25 x 20µL samples      |
|---|-------------------------|------------------------|---------------------|----------|---------------------------------|
| Water                                     | 125µL                   | 162.5µL                | X                   | =        | 25 x 125µL = 3.125mL            |
| 20X DNA Extraction Buffer (PN 310 00 40)  | 25µL                    | 32.5µL                 | X                   | =        | 25 x 25 = 625µL                 |
| 20X Sarcosyl Solution (PN 310 00 30)      | 25µL                    | 32.5µL                 | X                   | =        | 25 x 25 = 625µL                 |
| 8M Guanidine Hydrochloride (PN 310 00 20) | 75µL                    | 97.5µL                 | X                   | =        | 25 x 75 = 1.875mL               |
|   | <b>0.05-50µL sample</b> |                        |                     |          | 6.25mL 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            | 242.5µL       | X                   | =              | 25 x 242.5µL = 6062.5µL       |
| Ethanol          | 242.5µL       | X                   | =              | 25 x 242.5µL = 6062.5µL       |
| 5M NaCl Solution | 14.6µL        | X                   | =              | 25 x 14.6µL = 365µL           |
|                  |               |                     |                | Ethanol-Saline Wash - 12.49mL |

**1X DNA Elution Buffer:** Use immediately

| 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 = 1.125mL                               |
| 10X DNA Elution Buffer (PN 300 00 01) | 1µL             | 2µL             | 3.5µL           | 5µL             | 7.5µL           | 10µL             | 5µL x 25 = 1.25µL                                 |
|                                       |                 |                 |                 |                 |                 |                  | 1.25mL 1X DNA Extraction Buffer                   |

\*\*\* Extraction \*\*\*

**Step 2.** Dispense 2X DNA Extraction Buffer into a microfuge tube.

- ❖ For samples less than 50µL blood sample, dispense 250µL
  - ❖ For samples 50µL - 200µL blood sample, dispense 325µL
- Add sample directly to the buffer. Mix by pipetting or vortex.

**Step 3.** Raise the total volume in the tube with Water as follows:

- ❖ For samples less than 50µL, raise to 500µL,
- ❖ For samples 50µL - 200µL, raise to 650µL.

For example:

|                             |       |       |       |       |       |
|-----------------------------|-------|-------|-------|-------|-------|
| <b>Blood Sample Volume</b>  | 2µL   | 25µL  | 50µL  | 150µL | 200µL |
| <b>2X Extraction Buffer</b> | 250µL | 250µL | 325µL | 325µL | 325µL |
| <b>+ Water</b>              | 248µL | 225µL | 275µL | 225µL | 100µL |
| <b>= Volume</b>             | 500µL | 500µL | 650µL | 650µL | 650µL |

Mix well by vortex.

**Step 4.** Add Savinase™ and vortex sample.

- ❖ For samples less than 50µL, add 50µL Savinase
- ❖ For samples 50µL - 200µL, add 100µL Savinase

Incubate 0.05 - 10µL input blood samples at 56°C for at least one hour.  
Incubate larger samples at 56°C for at least 8 hours, preferably overnight

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

The supernatant should be colorless to transparent pale green in color.

Transfer<sub>7</sub> to a new silanized microfuge tube.

- ✓ User may consult the rotor speed conversion chart (nomogram) 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 10µL of PrepParticle Suspension (PN 100 00 00) and mix by vortex.

- ❖ If less than 3µL whole blood was added in Step 2, only 5µL PrepParticle Suspension are required. However, a 5µL pellet is very small and use of up to 10µL PrepParticle Suspension eases pellet visualization in Step 9.

**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 650µL 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.**

*\*\*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 at room temperature.

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

**Step 11.** To elute DNA from the PrepParticles, add 10 - 100µL of 1X DNA Elution Buffer directly to the surface of the pellet using the chart below as a general guideline to desired concentration. *Do not agitate the tube's contents* but allow 1X DNA Elution Buffer to rehydrate the pellet for 15min at 56°C

|            |    |    |    |    |    |    |    |    |    |     |     |     |     |     |
|------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|
| µL Blood   | 1  | 2  | 10 | 10 | 25 | 25 | 50 | 50 | 75 | 100 | 100 | 150 | 150 | 200 |
| µL Buffer  | 10 | 10 | 25 | 50 | 25 | 50 | 25 | 50 | 50 | 50  | 100 | 50  | 100 | 100 |
| ng DNA /µL | 20 | 40 | 8  | 4  | 20 | 10 | 40 | 20 | 30 | 40  | 20  | 60  | 30  | 40  |

- ✓ Calculations based on convention that 1µL whole human blood contains 3000 to 5000 nucleated cells and thus, 20 - 30ng DNA/µL. Resulting DNA eluates will be ready for PCR analysis as long as the DNA preparation does not exceed 20% of the total reaction volume. For buffy coat fractions, assume nucleated cell content per unit volume to be 3 to 5 times greater than that of whole blood, thus 1µL buffy coat fraction holds approximately 60 to 150ng DNA.

**Step 12.** After 15min rehydration, vortex 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 input blood volume in Step 2.) the more heating and agitation cycles will be required to resuspend the pellet since DNA is a very cohesive species. A 200µL whole blood sample may require as many as 5 agitation and heated incubation cycles for resuspension and optimal DNA yield. The process of pellet resuspension, with concomitant release of DNA into the elution buffer, may be expedited by gentle pipeting with a P-200 tip. Avoid generating foam and note that this added measure may result in diminished DNA yield proportional to residual volume loss in the pipet tip itself.

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 eluate from 200µL input whole blood may be slightly yellow due to abundant proteins' interaction with PrepParticles during the early lysis step.

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°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.**

|        | 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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