ARGYLLA DNA XtremeXtract Kit, Pepsin pan NA XX12-020.01

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY

Circulating Cell-free DNA/RNA in Plasma, Fifty (50) 0.5 mL samples

This dual protease protocol describes how to isolate and purify DNA and RNA from blood plasma and serum. This protocol has multiple utilities: (1) small and/or degraded DNA and RNA and (2) larger fragments of DNA suitable for PCR. Frozen or fresh plasma may be used. Emphasis is placed on denaturization of nucleases and digestion as soon as possible. The "pan NA" edition of the XtremeXtract kit uses nanoparticles that isolate the very small as well as genomic size fragments of DNA and miRNA.

The general strategy is as follows:

(1) Alkaline Digest for 1 hour:

- Plasma is added to urea for a final concentration of 6M in a micro-centrifuge tube
- ß-mercaptoethanol is added at 1% per volume (~143 mM) and the alkaline protease Savinase™

(2) Acidic Digest for 1 hour:

- The Savinase digest is acidified to pH 4 by adding 0.5 volumes of 170 mM HCl.
- The acidic protease Pepsin™ is added. Depurination is minimized at this pH and temperature.

(3) Argylla PrepParticles are added to capture the DNA and RNA.

(4) PrepParticles are rinsed to eliminate trace contaminants.

(5) Nucleic acids are eluted to user desired concentration.

Further size selection protocols are available. Unlike previous Argylla protocols, alcohols are not required for the precipitation. Like all Argylla protocols, the hazardous materials phenol and chloroform are not required.

www.Argylla.com/protocols

REAGENTS & CONSUMABLES

The Argylla ExtremeXtract Kit includes:

Component	Part Number	Volume	Storage
PrepParticle Suspension	100.00.00-L	2.5 mL	Store in darkness & at room temp
Elution Buffer pH 9 (100 mM $Na_2B_4O_7$)	200.01-1.5mL	1.5 mL	Store at room temperature
pH 4 Rinse (200x)	206.00-1mL	1.0 mL	Store at 4°C
Savinase™ *	311.00.01-S	25 mL	Store at 4°C in dark
Urea	401.01-20g	20 g	Store at room temperature in dry place
5M Sodium Chloride (NaCl)	501.00-3mL	3 mL	Store at room temperature
1M Tris-HCl, pH 8.0	203.00-1mL	1 mL	Store at room temperature
Pepsin	302.02-2g	2 g	Store at 4°C in dark

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

70% 2PrOH Isopropanol Rubbing Alcohol	Any drug store or chemical supply brand
ß –ME, 2 –mercaptoethanol	Amresco M131-100ML
1M HCl, Hydrochloric Acid	Amresco #E447
DNA grade H_2O	

*The following are registered trademarks: Savinase™(Novozyme Corp.),

Note:
All Argylla protocols recommend using quality silanized plastic labware such as pipette tips and tubes, which 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 kits can process and deliver as little as 100 picograms of DNA.

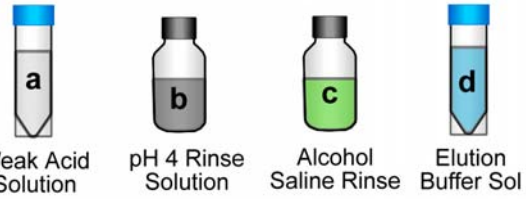
Argylla method for Isolating DNA/RNA from Plasma

See protocol for complete instructions

Preparation - Step 1

Prepare solutions a,b,c,d

1



Alkaline Digest - Steps 2-3

Add: Urea, ß-ME, Plasma, Savinase

2

3 Digest for 1 hour

Acidic Digest - Steps 4-5

4 Add Pepsin to (a)Weak Acid in a new tube

Add PrepParticles to Pepsin/acid Solution

Incubate for 30 mins

Centrifuge

Transfer Pepsin/acid solution without the pellet to a new tube

discard the pellet

Pepsin/Acid Solution

Add Pepsin/acid Solution to Alkaline Digest

5 Digest for 1 hour

Purification - Steps 5-10

6 Add PrepParticles

7 Incubate for 15 mins

8 Centrifuge

Discard supernatant Retain pellet

PrepParticle DNA pellet

9 Rinse with pH 4 rinse b

Disperse pellet by pipetting

Centrifuge

Discard supernatant Retain pellet

PrepParticle DNA pellet

10 Rinse with Alcohol Saline c

Disperse pellet by pipetting

Centrifuge

discard supernatant Retain pellet

(Repeat rinse Steps 9 and 10 if needed)

11 Evaporate alcohol and dry pellet

(DNA is stable and can be stored for future use at this point)

Dry PrepParticle DNA pellet

Elution and Concentration - Steps 11-12

12 Add Elution Buffer Sol d

Disperse and fully resuspend pellet by pipet or vortex

13 Centrifuge

Retain DNA Supernatant and Transfer to new tube

Discard spent PrepParticle pellet

DNA/RNA containing eluate

 www.argylla.com 1-800-234-6642

Instructions for Isolating DNA and RNA from blood plasma

*** Preparation ***

- Set a heat-block to 56^0C

Step 1. Prepare the following five solutions according to the number of samples to be processed.

(a) Weak Acid Solution
Prepare weak acid solution in a 50mL tube. Add in order listed:

1 M HCl Hydrochloric Acid	*To* 7.5 mL mark
DNA grade Water	*To* 45 mL mark

(b) pH 4 Rinse Solution 100mL for 100 samples.

DNA grade H_2O	95.5 mL
pH4 Rinse (206.00)	0.5 mL

Stable at room temperature for several months

(c) Alcohol Saline rinse
Add components in the order indicated. It is very convenient to make this in a 50 mL polypropylene tube.

5M NaCl Sodium Chloride (501.00)	1.5 mL
DNA grade H_2O	*To* 12.5 mL mark
1M Tris-HCl pH 8 (203.00)	0.5 mL
70% 2PrOH Isopopranol Alcohol	*To* 50 mL mark

(d) Elution Buffer Solution 5mL for 50 samples.

DNA grade H_2O	4.5mL
Elution Buffer pH 9, (200.01)	0.5mL

Stable at 4^oC for several months

*** Extraction ***

Step 2.
i)To maintain a constant volume of digest in an extraction tube, add 0.27g Urea for every 0.75 mL of the final volume of plasma. Add 7.5 µL ß-ME. Add fresh plasma to the final volume (0.75 mL) and 15µL Savinase for every 0.75 mL of plasma/urea solution. (*See Application Note *2i)*
Use the table below as a guide.

Component	1.5mL tube	2 mL tube	3mL tube	5mL tube
Urea	0.27 g	0.47g	0.648g	1.24 g
ß-ME	7.5µL	13.3µL	18µL	34µL
Plasma, add	*to* 0.75mL mark	*to* 1.3mL mark	*to* 1.8mL mark	*to* 3.4mL mark
Savinase	15µL	26.6 µL	36µL	68µL

ii) To digest the entirety of a given volume of plasma (frozen or liquid), add 0.31g Urea, 13µL ß -ME, and 26µL Savinase™ for every mL of plasma. Use table in step 2i) to estimate volume of tube needed. (*See Application Note *2ii)*

- As a negative control, it is recommended to perform a mock extraction of 0.5 mL phosphate buffered saline (PBS).

Step 3. Mix thoroughly. Digest with Savinase™ for one hour at 56^oC.

Step 4. **Pepsin/acid Preparation** (to remove trace nucleic acids)

- Calculate total volume of plasma to be processed.
- Measure 0.75 mL of (a)Weak Acid Solution for every mL of plasma to be processed into a separate tube.
- add 20mg Pepsin per mL of weak acid solution, mix, incubate 15 minutes at room temperature.
- add 60µL PrepParticles (100-00-00) per mL of pepsin/acid solution, mix and incubate 15 minutes at room temperature.
- Centrifuge to pellet the PrepParticles.
    - small volumes in microfuge tubes: 8000g for 5 minutes
    - large volumes in 15-50mL tubes on a desktop swinging bucket centrifuge: 2-4000g for 15-30 minutes
- Carefully decant or pipette pepsin/acid solution to another vessel leaving the pellet behind and undisturbed.

Step 5. Add 0.5 mL of Pepsin/acid Solution for every 1mL of plasma solution and Mix. Test the pH of the first tube, which should be 4-5. Adjust with 0.1 M HCl (or NaOH) as needed. (*See Application Note *4b)*.

- Digest with Pepsin for one hour at room temperature.

*** Purification ***

Step 6. Thoroughly agitate the PrepParticles (100-00-00) to an even suspension, free of aggregates, before each use. Add 10-20µL PrepParticles for every 0.75 mL of the digest.

Component	1.5mL tube	2 mL tube	3mL tube	5mL tube
PrepParticles	10µL	17µL	24µL	45µL

This protocol is scalable. PrepParticle volumes of 20µL or less per tube seem to work best.

Step 7. Incubate at room temperature for 15 min. with periodic vortexing to keep the particles in suspension.

Step 8. Centrifuge for 2 min. at 4000 x g. Discard the supernatant. **Retain the pellet**.

Step 9. Rinse the pellet with 0.5 mL (b) pH 4 Rinse Solution by very gently pipetting up and down three times. Centrifuge for 2 min. at 4000 x g. Discard the supernatant. **Retain the pellet**.

Step 10. Rinse the pellet with 0.5 mL (c) Alcohol Saline Rinse by very gently pipetting up and down three times. Centrifuge for 2 min. at 4000 x g. Discard the supernatant. **Retain the pellet**.

➢ **Rinsing Steps 9 and 10** One may insert extra rinse steps to remove contaminating hemoglobin.

Step 11. Pulse centrifuge to drive residual 2PrOH to bottom of tube. Remove supernatant with 20 µL pipette tip. Evaporate residual 2PrOH in uncapped tube at 56°C. (Small hairline cracks appearing in pellet is a good indicator that the pellet is dry). **Retain the pellet.**

➢ At this step the DNA is stable and can be stored at room temperature for long periods of time for later use.

*** Elution and Concentration ***

Step 12. Add 25-100 µL (d) Elution Buffer Solution to elute DNA from the pellet. Allow the pellet to rehydrate for 5-15 min at 56°C. Then gently pipette or vortex to fully disrupt and resuspend the pellet to a slurry. No particle aggregates should be visible in suspension. Large amounts of genomic sized DNA may require more time.

Step 13. Centrifuge the suspension for 5 min. at 8,000 x g to pellet the spent PrepParticles from the DNA-containing solution. **Retain the Supernatant**, which is the final DNA/RNA-containing eluate. Transfer to a new tube.

**Application notes

The user is given two choices:

***Step 2i) Constant volume in extraction tube** An example of this would be digesting as much as a 2mL flat walled polypropylene tube could hold without spilling any.

***Step 2ii) Digestion of entire amount of plasma.** An example of this would be extraction of the entirety of 0.85 mL of a frozen tube of plasma. The user need not be concerned with slight changes in volume of frozen plasma upon thawing.

***Step 4a)** The porcine pepsin contains ~100ng/mg RNA and ~14ng/mg DNA. It should be highly degraded after 30 mins in weak acid. To remove trace nucleic acids and other contaminants, add 60µL PrepParticles per mL of pepsin/acid solution.

***Step 4b)** When adding one part of Pepsin in weak acid to two parts Savinase, digestion of plasma in 6M urea, the pH comes very close to pH 4. PHydrion 3-5.5 pH paper (Cole-Palmer EW-59200-26) was used in development.