ARGYLLA DNA nanoPurify Kit

Mini Scale, A10M011 (50 Lonza gel recoveries)
Standard Scale, A10M013 (125 Lonza gel recoveries)

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY

Lonza Recovery Well Samples

This protocol describes how to isolate and purify Nucleic Acid bands from the recovery wells of Lonza gels. DNA separated from the Lonza proprietary fluorescent DNA so that the 260 nm absorbance can be measured with a NanoDrop spectrophotometer. This protocol will provide recovery and purification over a wide range of humidity, and heating variables that may be encountered in the use of Lonza recovery gels.

Elution may be scaled to user determined Nucleic Acid concentrations. Additional concentration steps are typically not needed.

A variety of DNA elution solutions may be used depending on downstream applications. If using the Argylla 1x Elution buffer, DNA is ready for PCR analysis as long as the DNA preparation does not exceed 10% of the polymerase chain reaction volume. The protocol is flexible and readily scaled.

Additional protocols for this kit to extract DNA from the following sources can be accessed on our website:
* Dried Blood Spots on Fibers * Buccal Wash * Samples Dried on Swabs * Flash Frozen Tissue Thin Sections Formalin-Fixed Paraffin-Embedded Tissue (FFPE) * Cell-Free DNA from Serum (beta)

www.Argylla.com/protocols

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
70% isopropanol (2PrOH)	Any drug store variety
Water, DNA-Grade	Fisher Scientific No. BP2470-1
5M Sodium Chloride (NaCl)	Sigma-Aldrich No. S5150
Costar Prelubricated Microfuge Tubes (silanized), 1.7mL	Costar no. 3207

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Instructions for Isolating DNA from Lonza Recovery Gels

*** **Preparation** ***

- Set heat block to 52^{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 solution according to the number of samples to be processed.

1x Elution Buffer 5 mL

Add:	
DNA grade water	4.5 mL
Argylla 10x Elution Buffer PN 300 00 01	0.5 mL

*** **Purification** ***

Step 2. Collect samples recovered from Lonza recovery wells in 0.5 mL polypropylene tubes. Note the approximate volume recovered.

Step 3. Add 10 µL Argylla PrepParticles. Vortex for 5 sec to mix.

Step 4. For the total volume of Recovery + PrepParticle volume in each tube, add 2 x that amount of anhydrous EtOH [a]. Vortex for 5 secs to mix. (See footnote A)

Step 5. Allow particles to settle out of suspension for 5 minutes at room temperature. Vortex for 5 secs. Wait another 15 minutes.

Step 6. Centrifuge at 2000xg for 5mins. Discard supernatant.

Step 7 Pulse centrifuge to collect residual EtOH/water to the bottom of the tube. Remove with a 20 µL pipette tip.

Step 8. Evaporate residual EtOH/water at 52°C. When enough EtOH/water has evaporated, the surface of the pellet will resemble pieces of a puzzle. The pellet may also detach from the tube and roll up into a tube. Expect to see a large decrease in the apparent volume of the pellet.

Step 9. Add 10µL 1x Elution buffer (see footnote B) to each sample.

Step 10. Allow pellets to rehydrate for 5 mins at room temperature. Vortex. Wait 15 mins.

Step 11. Gently pipette up and down 5 times to break up the particles. Continue until the particles are in close to their original colloidal suspension or are less than 0.1mm in diameter.

Step 12. Centrifuge at 10,000xg for 5 minutes. Retain the supernatant.

Footnotes

A) The protocol has been rigorously tested with two volumes of anhdydrous EtOH. Two volumes of absolute EtOH and two volumes of isopropanol (2PrOH) also work.

B) Other alternatives that have been tested include 20 mM sodium phosphate pH 7.5 and 20 mM $Na_2B_4O_7$. Both of these solutions will also elute DNA. If using Argylla Elution buffer, sample volume should be less than 10% of total PCR reaction volume. 1X Elution Buffer contains: 0.1 mM EDTA, 0.001% Tween 20, and 10mM sodium tetraborate.

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