# ARGYLLA DNA nanoPurify Kit

Mini Scale, A10M011 (4 - 25nmol purifications )
Standard Scale, A10M013 (10 - 25nmol purifications)

ARGYLLA TECHNOLOGIES DNA EXTRACTION BY PREPPARTICLE NANOCHROMATOGRAPHY

## Purification of Oligonucleotides

This protocol describes how to purify oligonucleotides of contaminants associated with their synthesis. Our product is an inexpensive way to achieve HPLC achieved purity. The purification can also be performed in the tubes that the oligonucleotides are sold.

The true utility of Argylla's nanoPurify Kit is that it can be scaled to only small amounts of oligonucleotide primers on an as-needed basis. It is a very good product to have on your shelf to be used when you find your favorite oligonucleotide vendor's "standard desalting" just wasn't good enough. Our product has been thoroughly tested on <20mer oligonucleotides giving our PrepParticles an advantage over many spin columns.

Our product may also be used to purify unbound fluorescent labels from sample DNA for DNA microarrays.

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**

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### REAGENTS & CONSUMABLES

**The Argylla DNA nanoPurify Kits (mini and standard) include:**

| | | |
|---|---|---|
| PrepParticle Suspension | PN 100 00 00-S, 0.5mL<br>PN 100 00 00-L, 2.5mL | Store in darkness & at room temp |
| 20X Lithium Chloride Solution | PN 300 00 10-S, 1.25mL<br>PN 300 00 10-L, 8.75mL | Caustic; eye, skin & respiratory irritant |
| 20X Sarcosyl™ Solution | PN 310 00 30-S, 1.25mL<br>PN 310 00 30-L, 6.25mL | Irritant to eye, skin, respiratory system |
| 10X DNA Elution Buffer | PN 300 00 01-S, 0.5mL<br>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 |
| 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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## Instructions for Purifying oligonucleotides

### *** Preparation ***

- Set heat block to 52-56°C
- It is important to thoroughly agitate the PrepParticle Suspension so that all aggregates are resuspended before each use.

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

**Ethanol Saline Rinse,** 100mL, (0.5 to 1.5mL per sample)

| Order of addition: | | |
|---|---|---|
| | 5M NaCl | dry Salt |
| 1. NaCl: (use 5M NaCl or dry salt) | 3.2mL | 0.94 g |
| 2. DNA Grade Water | 21.8mL | 25mL |
| 3. 100% Ethanol (EtOH) | 75mL | 75mL |

Cool to 4°C after preparation

### *** Purification ***

**Step 2.** If the oligonucleotides were sold in the dry form, add water to give a final concentration of 200 µM. Use this example of "dimensional analysis" or the "unit factor label method" for easy calculation to achieve a concentration of 200µM, or 200 x $10^{-6}$ moles per liter from 25 x $10^{-9}$ moles or 25nmoles of oligonucleotide.

$$\frac{\text{liter}}{200 \times 10^{-6}\ \text{moles}} \times \frac{25 \times 10^{-9}\ \text{moles}}{} = 0.000125 \text{ liters or } 125\mu L$$

Record your **starting volume** in the volume workseet.

| **Oligo Start Volume** | PrepParticles x 0.9 | 20x LiCl x 0.10 | Isopropanol x 7.0 |
|---|---|---|---|
| µL | µL | µL | µL |
| µL | µL | µL | µL |
| µL | µL | µL | µL |

**Step 3.** Multiply the **starting volume by 0.9**. Add this volume of µL Argylla PrepParticles. Vortex for 5 sec to mix.

**Step 4.** Multiply the **starting volume by 0.10**. Add this volume of µL Argylla 20x LiCl. Vortex for 5 sec to mix.

**Step 5.** Multiply the **starting volume by 7.0**. Add this volume of µL isopropanol. Vortex for 5 sec to mix. Incubate at room temperature for 10 min with occasional vortexing.

**Step 6.** Centrifuge at 2000xg for 5 minutes. Discard the supernatant.

**Step 7** Add 0.5 mL of the 4°C Ethanol Saline Rinse to the pellets. Vortex well enough to break up the PrepParticle pellet (see footnote A).

**Step 8.** Centrifuge at 2000xg for 5 minutes. Discard the supernatant. Pulse centrifuge after discarding the supernatant. Remove additional Ethanol Saline Rinse with a 20 µL pipette tip.

**Step 9.** To achieve maximal recovery, incubate the uncapped tube at 52-56°C to evaporate residual Ethanol Saline Rinse. Small cracks in the pellet surface indicate dryness.

**Step 10.** Add TE or 10 mM Tris pH 8 to 0.9 - 1x the original volume of the 200 µM solution (see footnote B).

**Step 11.** Allow to rehydrate at 56°C for 5 minutes *without* vortexing. Incubate for an additional 10 minutes at 52-56°C with periodic vortexing.

**Step 12.** Centrifuge at 10,000xg for 5 minutes. **Retain the supernatant.** (See footnote C)

**Footnotes**

**A)** The oligo contaminant triethylamine (TEA) is more soluble in alcohol and water below 18°C. Try to keep the temperature in the 4-18°C range. *When removing unconjugated fluorescent labels form RNA or DNA, you might want to do your own solubility studies.*

**B)** *Water can be used for this application, but not recommended.* If using Argylla Elution buffer that is incidentally supplied with the nanoPurify kit, 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.

**C)** If the user is ambidextrous, the supernatant may be drawn up into a pipette tip with one hand and the PrepParticles rinsed out of the tube with DNA grade water with the other hand.

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