Isolating DNA From Fresh blood

Using the Gene-Aid Kit (Cat# GB100 or GB300) or similar:

Price/isolation:

With GB300 Ft 577.57

With GB100 Ft 642.3

Sample Preparation:

• Transfer up to 300 µl of blood to a 1.5 ml microcentrifuge tube.

I recommend using 300 µl for good quantity. If more DNA quantity is needed, use more than 300 µl (up to 1 ml) and place into a sterile 15 ml centrifuge tube (instead of 1.5 ml microcentrifuge tube).

I also recommend using anticoagulant here (I once tried without – no good!) (E.g., 15 %

ACD-A).

When doing mice genotyping: I use 20-30 µl blood in 300 µl PBS + 10 mmol EDTA (no nanodrop reading, but enough for PCR/qPCR).

• Add 3X the sample volume of **RBC Lysis Buffer**, then mix gently by inversion 3-4 times. **DO NOT VORTEX.**

The main component here is ammonium chloride (exact recipe unknown)

To make your own:

For a 10X RBS Lysis solution:

in 100mL miliQ to be stored at 4 degrees.

NH4Cl (ammonium chloride) - 8.02g

NaHCO3 (sodium bicarbonate) - 0.84g

EDTA (disodium) - 0.37g

• Incubate the tube for 10 minutes at room temperature.

If left for too long/high temp: ammonium chloride can breakdown leukocytes & lymphocytes too early.

• Centrifuge for 5 minutes at 3,000 x g then remove the supernatant completely.

(For our genotyping we don't always see a pellet)

• Re-suspend leukocyte pellet in 100 µl of **RBC Lysis Buffer**, then proceed with Cell Lysis.

Cell Lysis:

• Add 200 µl of **GB Buffer** *(from the KIT)* then shake the 1.5 ml microcentrifuge tube vigorously or vortex. (Optional: Proteinase K)

chaotropic salts are used in this GB buffer (Recipe unknown) Common chaotropic agents are phenol, ethanol, guanidine hydrochloride, urea, and lithium perchlorate

• Incubate at 60 °C for at least 10 minutes to ensure the sample lysate is clear.

During incubation, it helps if you invert the tube every 2-3 minutes (works without this).

This is a good time to preheat the required **Elution Buffer** (200 µl per sample) to 60 °C (for Step 4 DNA Elution). The Kit recommendation is to use 200 µl of elution buffer/ sample, if using smaller amounts of blood – use less buffer (we use 30 µl, which is about the minimum)

[Optional Step: RNA Degradation (If RNA-free gDNA is required, perform this optional step)

Following 60 °C incubation, add 5 µl of **RNase A** (10 mg/ml) to the clear lysate then mix by shaking vigorously.

Incubate at room temperature for 5 minutes.

Depending on what you will use the DNA for – you may need to degrade the RNA (particularly for NGS) (I have never used this step)

DNA Binding

• Add 200 µl of absolute ethanol (cold) to the lysate then immediately mix by shaking vigorously for 10 seconds (precipitation).

NOTE: If visible precipitate appears, break it up as much as possible with a pipette.

• Place a **GD Column** in a 2 mL Collection Tube.

• Transfer the mixture (including any precipitate) to the **GD Column** then centrifuge at 14-16,000 x g (I use 15,600 g) for 5 minutes.

• Discard the 2 ml Collection Tube then place the **GD Column** in a new 2 ml Collection Tube.

> The GD column is made up of a cotton mesh-like structure containing a glass fibre matrix. The glass fibre matrix is where the DNA will bind. Silica fibre matrix will work too.

Wash

• Add 400 µl of **W1 Buffer** to the **GD Column** then centrifuge at 14-16,000 x g (I use 15,600 g) for 30-60 seconds.

• Discard the flow-through then place the **GD Column** back in the 2 ml Collection Tube.

• Add 600 µl of **Wash Buffer** (make sure ethanol was added to the Wash Buffer (depending on kit)) to the **GD Column**.

> W1 and Wash Buffer seem to be very similar (for best results use both). The primary ingredient is ethanol (also commonly used 20 mM NaCl, 2 mM Tris-HCl) (full recipe unknown) which helps remove contaminants bound to the DNA.

• Centrifuge at 14-16,000 x g (I use 15,600 g) for 30-60 seconds then discard the flow-through.

• Place the **GD Column** back in the 2 ml Collection Tube.

• Centrifuge again for 3 minutes at 14-16,000 x g (I use 15,600 g) to dry the column matrix.

- Extra drying step by changing tube and re-spin (to have the better 260/280 and 260/230 ratio)

DNA Elution

• Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube.

• Add 100 µl of pre-heated Elution Buffer (in 60 degree), TE or water to the CENTER of the column-matrix.

• Let stand for at least 3 minutes in RT to ensure the Elution Buffer, TE or water is completely absorbed (it can stay longer)

• Centrifuge at 14-16,000 x g (I use 15,600 g) for 30-60 seconds to elute purified DNA

If higher DNA yield is required, repeat DNA Elution step to increase DNA recovery (DO NOT increase the total elution volume to > 200 µl).

Minimum elution volume is 30 µl/elution (enough to wet the entire matrix).

For short term storage: store at 4 °C

For long term storage: store at -20 °C

Optional kiki : for further purification make a solution of 1 : 25 chloroform : sample, mix it without vortexing, and spin with 15600g for 15 min. The upper water-clear clear layer contains DNA

Isolating DNA From PLASMA and viral DNA and cell culture and mitochondrial

This GeneAid Kit also contains protocols for extracting genomic DNA from buffy coat blood (white blood fraction) and cultured cell samples as well as mitochondrial and virus DNA.

The Kit does not specify DNA isolation from Plasma, however; the protocol will be fairly similar to fresh blood DNA isolation (the RBC lysis and even perhaps cell lysis steps can be skipped).

Only small amounts of DNA can be found in blood plasma (not ideal for large quantity of DNA). The DNA here will be very fragmented (not suitable for many applications). Mainly used as a diagnostic tool (e.g., cancer).