TNArex Purification Kit Protocol – Whole Blood – DNA

Average Yield Range: Depending on sample

Cell Lysis

1. Add 300 µl of whole blood (or bone marrow) to a 1.5 ml microfuge tube containing 900 µl of **1X RBC Lysis Solution**. Incubate 10 minutes at room temperature and invert gently 10 times during the incubation.
   Note: For fresh blood, collected within 1 hour, increase incubation time to 3 minutes to ensure complete red blood cell lysis.
   Note: To prepare 1X RBC Lysis Solution, add 90 µl of 10X RBC Lysis Solution into 810 µl of sterile deionized water.
2. Centrifuge for 20 seconds at 13,000-16,000 rpm. Remove as much supernatant as possible with a pipette leaving behind the visible white cell pellet and about 10-20 µl of the residual liquid.
3. Vortex the tube vigorously for 10 seconds to resuspend the white cells in the residual liquid; this greatly facilitates cell lysis in Step 4 below. The white cell pellet should not be visible following vortexing.
4. Add 300 µl of **TNA Lysis Solution** to the resuspended cells and pipet gently up and down no more than 10 times to lyse the cells (alternatively, vortex for 10 seconds on high speed to mix).
5. For DNA isolation, proceed to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.

RNase Treatment

1. Add 1.5 µl of **RNase A Solution** to the cell lysate.
2. Mix the sample by inverting the tube 25 times and incubate at 37°C for 15 minutes.

Protein Precipitation

1. Cool sample to room temperature. Add 100 µl of **Protein Precipitation Solution** to the cell lysate. Vortex vigorously at high speed for 20 seconds to mix the **Protein Precipitation Solution** uniformly with the cell lysate. Incubate on ice for 5 minutes.
2. Centrifuge at 13,000-16,000 rpm for 10 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not tight, repeat centrifugation.

TNA Precipitation

1. Transfer 300 µl of supernatant containing the TNA (leaving behind the pellet) into a clean 1.5 ml tube containing 300 µl of **100% Isopropanol**. Invert tube gently 50 times.
2. Centrifuge at 13,000-16,000 rpm for 5 minutes. The DNA will be visible as a small white pellet.
3. Discard supernatant and drain tube briefly on a clean absorbent paper. Carefully pour off isopropanol.
4. Add 300 µl of **70% Ethanol** and centrifuge at 13,000-16,000 rpm for 5 minutes. Carefully

pour off ethanol. *Pellet may be loose so pour slowly and watch pellet.*

5. Invert and drain the tube on clean absorbent paper. Allow to air dry for 15 minutes.

TNA Hydration

1. Add 40-50 µl of **TNA Hydration Solution**.
2. For DNA hydration, incubate at 65 °C for at least 25 minutes. Gently tap tube every 15 minutes. Store DNA sample at -20°C.