TNArex Purification Kit Protocol - Fresh/Frozen Animal Tissue

Expected Yield Range: 2-15 µg (DNA) or 0.5-10 µg (RNA)

Cell Lysis

1. Dissect tissue sample quickly and freeze in liquid nitrogen. Store at –80°C. Fresh tissue may also be used. Work very quickly and keep tissue on ice at all times including when tissue is being weighed.
2. Add 5-10 mg (0.005-0.01 g) of frozen tissue or fresh tissue to a 1.5 ml microfuge tube containing 300 µl of **TNA Lysis Solution**, remove it from ice and homogenize thoroughly using a microfuge tube pestle. Place sample back on ice until next step.
3. Incubate lysate at 55°C for 15-45 minutes. If maximum yield is required, 1.5 µl of **Proteinase-K Solution** (20 mg/ml) may be added to the lysate. Mix by inverting and incubate at 55°C for 15-45 minutes. If possible, invert tube during the incubation.
4. For DNA isolation, proceed to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.
5. For RNA isolation, cool sample to room temperature. Proceed to **Modified Protein Precipitation Solution** 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 x g for 10 minutes. The precipitated proteins will form a tight pellet. If the protein pellet is not tight, repeat centrifugation.

Modified Protein Precipitation

1. Add 100 µl of **Protein Precipitation Solution** to the cell lysate.
2. Invert tube gently 10 times and incubate on ice for 5 minutes.
3. Centrifuge at 13,000-16,000 x g for 10 minutes. The precipitated protein 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 x g 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 x g 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 RNA and TNA hydration, incubate on ice for at least 30 minutes. For DNA hydration, incubate at 65 °C for at least 25 minutes. Gently tap tube every 15 minutes. Store RNA/TNA sample at -80°C and DNA at -20°C.