TNArex Purification Kit Protocol – DNA and RNA Viruses

Cell Lysis

1. Add 100 µl body fluid (*e.g.*, cerebrospinal fluid, plasma, saliva, serum, sputum, synovial fluid, urine, whole blood, milk) to a sterile 1.5 ml microfuge tube containing 500 µl **TNA Lysis Solution**. Pipet up and down several times to mix thoroughly.
   Note: If the sample has a high protein content, 50 µl body fluid may be added to 550 µl **TNA Lysis Solution**.
2. Add 3 µl of **Proteinase K** (20 mg/ml), mix by inverting and heat to 55°C for 15 minutes for complete lysis. Alternatively, for maximum yield, incubate at 55°C for 1 hour to overnight.
3. For DNA isolation, process to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.
4. For RNA isolation, cool sample to room temperature. Proceed to **Modified Protein Precipitation Solution** and **TNA Precipitation**.

RNase Treatment

1. Add 3 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 for 5 minutes.
2. Add 200 µl **Protein Precipitation Solution** to the lysate.
3. Vortex sample at high speed for 20 seconds to mix the **Protein Precipitation Solution** uniformly with the lysate.
4. Place sample into an ice bath for 5 minutes or in 4°C for 10 minutes.
5. Centrifuge at 13,000-16,000 x g for 10 minutes. The precipitated proteins should form a tight pellet.
   Note: if body fluid has high lipid content, particulates may stay near top of tube; see alternative transfer method in Step 1 below in **TNA Precipitation**.

Modified Protein Precipitation

1. Cool sample to room temperature for 5 minutes.
2. Add 200 µl **Protein Precipitation Solution** to the lysate.
3. Invert tube for 10 times to mix the **Protein Precipitation Solution** uniformly with the lysate.
4. Place sample into an ice bath for 5 minutes or in 4°C for 10 minutes.
5. Centrifuge at 13,000-16,000 x g for 10 minutes. The precipitated proteins should form a tight pellet.
   Note: if body fluid has high lipid content, particulates may stay near top of tube; see alternative transfer method in Step 1 below in **TNA Precipitation**.

TNA Precipitation

1. Transfer 780 µl of the supernatant containing the DNA (leaving behind the precipitated protein pellet) into a clean 1.5 ml microfuge tube containing 780 µl **100% Isopropanol** (2-propanol). Alternatively, if particulates are present, transfer supernatant using a pipet so that particulates are excluded. If the DNA yield is expected to be low (<20 µg), add a DNA carrier such as **Glycogen** (1 µl **Glycogen Solution**, 20 mg/ml, per 600 µl **Isopropanol**).
2. Mix the sample by inverting gently 50 times and keep tube at room temperature for at least 5-10 minutes.
3. Centrifuge at 13,000-16,000 x g for 7 minutes. The DNA may or may not be visible as a small white pellet, depending on yield.
4. Pour off the supernatant and drain tube briefly on clean absorbent paper. Add 780 µl **70% Ethanol** and invert the tube several times to wash the DNA pellet.
5. Centrifuge at 13,000-16,000 x g for 7 minute. Carefully pour off the ethanol. *Pellet may be loose so pour slowly and watch pellet.*
6. Invert and drain the tube on clean absorbent paper and allow to air dry 15 minutes.

TNA Hydration

1. Add 20 µl of **TNA Hydration Solution** (20 µl will give a concentration of 100 ng/µl if the yield is 2 µg DNA).
2. Rehydrate DNA by incubating at 4°C for 30 minutes. Rehydrate RNA by incubating at 4°C for 1 hour. If possible, tap tube periodically to aid in dispersing the TNA.
3. Store both DNA and RNA at -80°C for long-term storage.