TNArex Purification Kit Protocol – 0.5-1.0 Million Cells

Average Yield Range: 5-10 µg of DNA/RNA

Cell Lysis

1. Add 0.5-1.0 million cells suspended in balanced salt solution or culture medium into a 1.5 ml microfuge tube on ice.
2. Centrifuge at 13,000-16,000 x g for 5 seconds to pellet cells. Remove supernatant with a pipette leaving behind visible cell pellet and approximately 10-20 µl of the residual liquid.
3. Vortex the tube to resuspend the cells in the residual supernatant (invert tube to check that the cell pellet has disappeared completely). This greatly facilitates cell lysis in Step 4.
4. Add 300 µl of **TNA Lysis Solution** to the resuspended cells and pipet up and down no more than three times to lyse the cells. Alternatively, cultured cells that adhere to culture plates or flasks may be lysed directly on the plate by first removing the culture media and then adding 300 µl of TNA Lysis Solution. Swirl solution to cover entire plate and draw lysed cells up and down in a pipette three times before removing to a 1.5 ml microfuge tube.
5. For DNA isolation, proceed to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.
6. 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.