TNArex Purification Kit Protocol - Gram Positive & Gram Negative Bacteria

Average Yield Range: 10-35 µg (DNA) or 10-30 µg (RNA)

Materials to be supplied by the user

· 50 mM EDTA (pH 8.0) (for gram positive bacteria)
· 10 mg/ml lysozyme (for gram positive bacteria)
· 10 mg/ml lysostaphin (for gram positive bacteria)

Cell Lysis

1. Add 0.5 ml of an overnight culture to a 1.5 ml microfuge tube.
2. Centrifuge at 13,000-16,000 x g for 2 minutes to pellet the cells. Remove the supernatant.
   Note: For Gram Positive Bacteria, proceed to Step 3. **For Gram Negative Bacteria go directly to Step 6.**
3. Resuspend the cells thoroughly in 240 µl of 50 mM EDTA.
4. Add the appropriate lytic enzyme(s) to the resuspended cell pellet in a total volume of 60 µl and gently pipet to mix. The purpose of this pretreatment is to weaken the cell wall so that efficient cell lysis can take place.
   Note: For certain *staphylococcus* species, a mixture of 30 µl of 10 mg/ml lysozyme and 30 µl of 10 mg/ml lysostaphin is required for efficient lysis.
5. Incubate the sample at 37°C for 30-60 minutes. Centrifuge for 2 minutes at 13,000-16,000 x g and remove the supernatant.
6. Add 300 µl of **TNA Lysis Solution**. Gently pipet until the cells are resuspended.
7. Incubate at 80°C for 5 minutes to lyse the cells; then cool to room temperature.
8. For DNA isolation, proceed to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.
9. 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.
2. 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.
3. 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 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.