TNArex Purification Kit Protocol - Yeast

Average Yield Range: 3-6 µg (DNA) or depending on sample (RNA)

Materials to be supplied by the user

· 20 mg/ml lyticase
· 50 mM EDTA (pH 8.0)

Cell Lysis

1. Add 0.5 ml of a culture grown for 20 hours in YPD broth to a 1.5 ml centrifuge tube.
2. Centrifuge at 13,000-16,000 rpm for 2 minutes to pellet the cells. Remove the supernatant.
3. Resuspend the cells thoroughly in 293 µl of 50 mM EDTA. Add 7.5 µl of 20 mg/ml lyticase and gently pipet 4 times to mix.
4. Incubate the sample at 37°C for 30-60 minutes to digest the cell wall. Cool to room temperature.
5. Centrifuge the sample at 13,000-16,000 rpm for 2 minutes and then remove the supernatant.
6. Add 300µl of **TNA Lysis Solution** to the cell pellet and gently pipet to mix.
7. For DNA isolation, proceed to **RNase Treatment** followed by **Protein Precipitation** and **TNA Precipitation**.
8. 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 rpm 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 rpm 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 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 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.