DNA Isolation Protocol (DNAzol)

Reagents and Materials:

- Eppendorf tube (1.5 ml)
- Pipettes and tips
- DNAzol reagent (Thermo Scientific)
- 100% Ethanol (Reanal)
- 75% Ethanol (Reanal)
- 8mM NaOH (Reanal)
- 0.1M HEPES (Sigma-Aldrich)

Isolation:

1. Cell pellets or Suspensions: Add 1 ml of DNAzol® Reagent to 1-3 × 10^7 cells, either in pellet or in suspension (volume < 0.1 ml). Lyse the cells by gentle pipetting. Mix DNA solutions by inversion; avoid shaking or use of a Vortex for mixing.
2. Precipitate DNA from the lysate/homogenate by the addition of 0.5 ml of 100% ethanol per 1 ml of DNAzol® Reagent used for the isolation.
3. Mix samples by inversion and store them at room temperature for 1-3 min. DNA should quickly become visible as a cloudy precipitate.
4. Sediment the homogenate for 1 min at 4,000 × g at 4 °C.
5. Remove the supernatant by pipetting.
6. Wash the DNA precipitate with 0.8-1.0 ml of 75% ethanol. At each wash, suspend the DNA in ethanol by inverting the tubes 3-6 times.
7. Sediment the homogenate for 1 min at 4,000 × g at 4°C.
8. Remove the supernatant by pipetting.
9. Repeat steps 6-8 twice.
10. Store the tubes vertically for 0.5-1 min to allow the DNA to settle to the bottom of the tubes and remove ethanol by pipetting.
11. Air dry the DNA by storing it in an open tube for 5-15 seconds after removing the ethanol.
12. Dissolve the DNA in 100 µl 8 mM NaOH by slowly passing the pellet through a pipette tip.
13. After DNA is solubilized in 8 mM NaOH, adjust the DNA solution to pH 7.8 by the addition of 11.7 µl 0.1M HEPES.

Quantitation of DNA with a Multi-Mode Microplate Reader:

- Drop 2 x 2 µl Elution Buffer (as Blank) on the first row and 2 x 2 µl DNA solution on the second row of microplate insert (Take 3).

- Measure the sample on 260/280nm using the Gene5. program /Nucleic Acid Quantification.

- Measure A_{260} and A_{280} of the DNA solution. Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA per ml.

  The A_{260}/A_{280} ratio of the isolated DNA is within the 1.9 - 2.1 range.