SOP for Extraction of Genomic DNA from Cultured Cells Using the DNAzol(R) Genomic DNA-Isolation Reagent

1. Scope

1.1 This procedure describes a method for the extraction of genomic DNA from cultured cells using the DNAzol(R) Genomic DNA-Isolation Reagent from GIBCO-BRL.

2. Reagents

2.1 DNAzol(R) Genomic DNA Isolation Reagent (#10503-027; GIBCO BRL)

2.2 99.5% Ethanol and 95% ethanol (#14713-95 and #14711-15; Nakarai Tesque)

3. Procedure for extraction of genomic DNA

3.1 For monolayer cultures, trypsinize cells to obtain a suspensions of 2-5 x 10^6 cells/mL. Transfer 1 mL of the suspension to a 1.5-mL centrifuge tube and pellet cells. For suspension cultures, transfer 1 mL of culture (~10^7 cells/mL) to a 1.5-mL centrifuge tube and pellet cells.

3.2 Add 1 mL of DNAzol(R) to the cell pellet and resuspend cells completely by pipetting.

3.3 Add 0.5 mL of 99.5% ethanol to the suspension of cells, invert the tube from five to eight times for thorough mixing. Allow the mixture to stand for one to three minutes at room temperature. You should see a cloudy precipitate of DNA in a uniform mixture of DNAzol(R) and ethanol.

3.4 Use the tip of a micropipette to collect the precipitated DNA by winding it around the tip and out of the solution and then transfer the DNA to another centrifuge tube that contains 1 mL of 95% ethanol. Stir rapidly to unwind the precipitated DNA from the tip of the micropipette.

3.5 Invert the tube three to six times to wash the DNA and then allow it to stand for one minute at room temperature. Carefully remove and discard the ethanol, taking care not to discard the DNA.

3.6 Add 1 mL of 95% ethanol and wash the pellet once more.

3.7 Remove ethanol completely and discard. Then add sterile water (50-100 μL) and redissolve the DNA by pipetteing.

3.8 Measure the optical density (O.D.) of the solution at 260 nm in a spectrophotometer and calculate the concentration of DNA from the following equation:

$$\text{Concentration of DNA } (\mu g/mL) = O.D._{260} \times 50$$

3.9 Dilute the solution of DNA with sterile water to a final concentration of 0.05 μg/μL, aliquot, label, and store at –20°C.