Protocol

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Nucleic Acid Precipitation from Dilute Solutions

Introduction

Glycogen is also an inert carrier used to increase nucleic acid recovery from alcohol precipitation. It is a preferred coprecipitant for solutions containing oligonucleotides or low concentrations of DNA or RNA, as it does not add exogenous nucleic acids like other coprecipitants, such as yeast RNA or tRNA. Glycogen, a highly purified branched chain carbohydrate, is insoluble in ethanol and isopropanol and forms a precipitate that traps nucleic acids. Upon centrifugation, the insoluble glycogen/nucleic acid precipitate forms a visible pellet that simplifies downstream sample processing.

Glycogen, 20 mg/ml, may be used for the recovery of oligonucleotides (>8 bases) and low amounts of nucleic acids (≥20 pg) from diluted solutions.

Materials

- Nucleic acid solution
- Glycogen, 20 mg/ml (GoldBio Catalog # G-090)
- 3M Sodium Acetate, pH 5.2
- Isopropanol or ethanol
- Nuclease-free water or TE Buffer, pH 8

Method

1. Add 1/10 volume of 3M sodium acetate, pH 5.2 to the nucleic acid in solution.

   Note: 1/10 volume of 2M sodium chloride or 5M ammonium acetate may be substituted.

2. Add Glycogen Solution, 20 mg/ml to final concentration of 0.05-1 µg/µl.

   Note: For oligonucleotides, use a final concentration of 1 µg/µl. For DNA or RNA, use a final concentration of 0.05-1 µg/µl of Glycogen Solution.

3. Add 1 volume of isopropanol to the solution. Mix gently.

   Note: 2.5 volumes of ethanol may be substituted.

   Note: Use ethanol for <200 bp fragments.

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4. Incubate for 1 hour at -20°C or for 30 minutes at -70°C.

   Note: Longer incubation times at lower temperatures may result in better recovery of nucleic acids.

5. Centrifuge for 15-30 minutes at 12,000 x g at 4°C. Discard the supernatant without disturbing the pellet.

6. Rinse the pellet with cold 70% ethanol.

7. Centrifuge for 5-15 minutes at 12,000 x g at 4°C. Discard the supernatant carefully to avoid disturbing the pellet.

8. Air dry the pellet for 5-10 minutes, being careful to not over-dry, which may render the pellet more difficult to dissolve.

   Note: Isopropanol may require longer drying time than ethanol.

9. Dissolve the nucleic acid pellet in nuclease-free water or TE Buffer, pH8.