Guide to Performing Serial Dilutions

Introduction

Serial dilution is a stepwise dilution of a substance in solution. It is commonly used in experiments requiring highly diluted solutions, such as assays and microbial counts.

What is a Serial Dilution?

- A method to systematically dilute a concentrated solution to achieve a range of concentrations.
- Each step involves diluting the previous solution, resulting in exponential dilution.

Applications

- Creating standard curves for assays
- Reducing concentration of samples to measurable levels
- Microbiological studies to estimate viable cell counts

Materials Needed

- Stock solution
- Solvent (e.g., distilled water)
- Pipettes and tips (appropriate volumes)
- Tubes or wells for dilution steps
- Vortex mixer (optional)

Basic Concepts

1. **Dilution Factor (DF)**
    - The ratio of the final volume to the aliquot volume.
    - **DF = Final Volume / Aliquot Volume**
2. **Total Dilution Factor**
    - The product of individual dilution factors up to that point.
    - **Total DF = $DF_1 \times DF_2 \times ... \times DF$□**
3. **Concentration Calculation**
    - **$C_2 = C_1 \times (V_1 / V_2)$**
    - Where:
        - C_1 = initial concentration
        - C_2 = final concentration
        - V_1 = aliquot volume
        - V_2 = final volume

Step-by-Step Procedure

Example: Performing a 1:10 Serial Dilution Across 5 Tubes

1. **Label Tubes**
    - Label tubes from 1 to 5.
2. **Add Diluent**
    - Pipette 9 mL of diluent into tubes 2 to 5.
3. **Prepare First Dilution**
    - Add 1 mL of stock solution to tube 1 (contains 9 mL diluent).
    - Mix thoroughly.
    - **Dilution Factor:** 1:10
4. **Perform Serial Dilutions**
    - **Tube 2:**
        - Transfer 1 mL from tube 1 to tube 2.
        - Mix thoroughly.
        - **Total Dilution Factor:** 1:100
    - **Tube 3:**
        - Transfer 1 mL from tube 2 to tube 3.
        - Mix thoroughly.
        - **Total Dilution Factor:** 1:1,000
    - **Tube 4:**
        - Transfer 1 mL from tube 3 to tube 4.
        - Mix thoroughly.
        - **Total Dilution Factor:** 1:10,000
    - **Tube 5:**
        - Transfer 1 mL from tube 4 to tube 5.
        - Mix thoroughly.
        - **Total Dilution Factor:** 1:100,000
5. **Optional: Plate or Use Dilutions**
    - Use the diluted solutions for your experimental application.

Tips for Accurate Dilutions

- **Mix Thoroughly:** Ensure the solution is homogenous before each transfer.
- **Change Tips:** Use a fresh pipette tip for each transfer to avoid cross-contamination.
- **Avoid Air Bubbles:** Ensure pipette tips are free of air bubbles when measuring liquids.
- **Use Calibrated Equipment:** Ensure pipettes are calibrated for accurate volume measurements.
- **Label Clearly:** Proper labeling prevents mix-ups during the procedure.

Calculating Dilution Factors for Non-Standard Dilutions

- **Custom Dilution Factor:**

    - If diluting by a factor other than 1:10, adjust volumes accordingly.
    - **Example:** For a 1:7.5 dilution:
        - Aliquot Volume (V_1): X mL
        - Diluent Volume (V_2 - V_1): (7.5 - 1) × X mL
        - **Total Volume (V_2):** 7.5 × X mL

Common Mistakes to Avoid

- **Incomplete Mixing:** Leads to inaccurate concentrations.
- **Incorrect Volume Transfers:** Double-check volumes before transferring.
- **Contamination:** Always use sterile techniques if required.

Applications in 96-Well Plates

- **High-Throughput Dilutions:**
    - Use multichannel pipettes for efficiency.
- **Plate Maps:**
    - Plan the layout of your dilutions on the plate.
- **Edge Effects:**
    - Be cautious of evaporation in outer wells; consider using edge wells as controls.