Phosphotungstic Acid Negative Stain Solution (2%)

Introduction

Negative staining, discovered by Hall, is a technique that contrasts with conventional positive staining. The principle involves using heavy metal salts to encase the sample, which has low electron density. This enhances the electron density of the surrounding medium, creating 'mass-thickness' differences between fine structures and increasing scattering/absorbing contrast. Consequently, the sample appears as a bright structure against a dark background. Common negative stains include Phosphotungstic Acid (PTA), Ammonium Molybdate, and India Ink. The most widely used one is 1-3% Phosphotungstic Acid .

Specifically, 2% Phosphotungstic Acid Negative Stain Solution is suitable for visualizing macromolecules, bacteria, viruses, protozoa, bacteriophages, organelles, nucleic acids, protein crystals, and other biological specimens. Following staining, the specimen exhibits a clear, bright appearance, while the background remains black.

Storage

Store at room temperature protected from light, stable for 1 year.

Protocol

1. Drop Method

    1) Concentrate the sample by low-speed centrifugation (2000 × g, 10 min) or other suitable methods to prepare a suspension with the desired concentration and purity.

    2) Apply a drop of the sample suspension directly onto a grid with a support film, and let it stand for 3-5 min.

    3) Remove excess liquid from the edge of the droplet using filter paper, and allow it to air-dry slightly.

    4) Apply a drop of 2% Phosphotungstic Acid Negative Stain Solution and let it stand for 2-3 min.

    5) Remove excess staining solution, allow the grid to air-dry completely, and then observe under the microscope.

2. Float Method

    1) Concentrate the sample by low-speed centrifugation (2000 × g, 10 min) or other suitable methods to prepare a suspension with the desired concentration and purity.

2) Float the grid with a support film on top of the sample droplet to adsorb the sample.

3) Float the grid on the surface of 2% Phosphotungstic Acid Negative Stain Solution for 1-2 min.

4) Remove excess staining solution, allow the grid to air-dry completely, and then observe under the microscope.

3. Staining Results

Specimen	Transparent bright
Background	Black

Note

1. The sample should be as fresh as possible.
2. The sample must be a homogeneous suspension with appropriate purity and concentration. Otherwise, specific and clear contrast reactions with the staining agent cannot be achieved.
3. For your safety and health, please wear lab coats and gloves during the experiment.
4. For research use only. Not to be used in clinical diagnostic or clinical trials.

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