Polybrene (10 mg/mL)

P1501912

Store at -20℃ long term (12 months). Store in the dark.

Introduction:

Polybrene (10 mg/mL) is a polycationic polymer, commonly used in DNA transfection experiments of mammalian cells to enhance the transfection efficiency of liposomes. Currently, Polybrene is widely applied in retrovirus-mediated gene transfection and lentivirus-mediated gene transfection. Its mechanism of action is presumably to neutralize the electrostatic repulsion between sialic acid on the cell surface and viral particles, thereby promoting adsorption.Polybrene is also a well-known anti-heparin agent (heparin antagonist) and is often used to produce non-specifically agglutinated red blood cells. In addition, it is frequently utilized in protein sequencing—low doses of Polybrene can significantly reduce polypeptide degradation during automated sequencing analysis. Adding Polybrene to PVDF membranes can also improve the membrane's affinity. This product is supplied in solution form. Specifically, the 10mg/mL packaged product is prepared with ultrapure water and sterilized using a 0.22μm filter membrane.

Instructions for Use:

1. Recommended Working Concentration:

- The optimal final concentration of Polybrene varies by cell line, typically ranging from 2–10μg/mL, with the most commonly used concentration being 5–8μg/mL. This can be determined by reviewing relevant literature or conducting preliminary experiments.

2. Usage Methods (For Reference Only):

Experiment 1: Retroviral Infection.

- Preparation of Recombinant Retrovirus Stock Solution: Add 5 mL of growth medium (containing 5% serum) to a 100 mm culture dish with a confluent monolayer of transfected retroviral packaging cells. After incubating for 24 hours, aspirate the culture medium and filter it through a 0.45 μm filter.
- Culture of Cells to Be Infected: Add 10 mL of complete medium to a 100 mm culture dish, adjusting the cell density to 5×10^5 cells per dish.
- Viral Infection: After culturing the cells for 24 hours, aspirate the complete medium. Infect the cells with 2 mL of viral supernatant containing Polybrene (or dilute the virus stock solution to 2 mL), ensuring the final concentration of Polybrene is 5–10 μg/mL. Incubate at 37°C for 3–6 hours.
- Collection of Viral Particles: Add 8 mL of complete medium. Three days after infection, lyse the cells using selection medium at a 1:5 ratio.

Experiment 2: Transfection.

- Culture cells in complete growth medium until the cell density reaches approximately 50%.

- After incubating the cells for 18–24 hours, prepare the DNA-medium-Polybrene mixture following these steps:

a) Add complete medium (2 mL for 60 mm culture dishes, 3 mL for 100 mm culture dishes) and prewarm it to 37°C.

b) Add 10 ng–10 μg of plasmid and mix gently.

c) Add Polybrene to a final concentration of 5–10 μg/mL and mix gently. The above components must be added in sequence.

- Remove the medium, add the DNA-medium-Polybrene solution to the cells, and incubate the cells at 37°C for 6–20 hours. Gently mix the cells approximately every 1.5 hours within the first 6 hours of cell culture.
- Remove the DNA-medium-Polybrene solution. Gently cover the cells with DMSO shock solution (15% DMSO in 1× HBSS) (3 mL for 60 mm culture dishes, 4 mL for 100 mm culture dishes). Gently shake the culture dish by hand for 10 seconds each time the solution is added to ensure uniform distribution of the liquid. Then incubate the cells at 37°C for 4 minutes.
- Immediately remove the DMSO shock solution and gently wash the cells twice with complete growth medium. Use 5 mL of culture medium per wash for 60 mm culture dishes, and 10 mL of culture medium per wash for 100 mm culture dishes.
- Add complete medium to the cells.
- Stable transfection: Remove the growth medium and lyse the cells with selection medium at a 1:5 ratio.
- Transient expression: Remove the growth medium and add fresh growth medium. Harvest the cells after 24–72 hours.

Matters needing attention:

1. To avoid repeated freeze-thaw cycles, it is recommended to appropriately aliquot the product upon receipt or after reconstituting it into a solution, then store it at -20°C.
2. Polybrene exhibits high toxicity to certain cell types (e.g., terminally differentiated neurons, DC cells). For first-time use, it is advisable to conduct a toxicity test beforehand.
3. This product may have certain toxic effects on the human body. Please take appropriate protective measures to avoid direct contact with the body or inhalation.
4. This product is intended solely for scientific research by professional personnel. It shall not be used for clinical diagnosis or treatment, nor for food or pharmaceutical purposes, and shall not be stored in ordinary residential premises.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.