Polybrene

Packing specification

Product number:IN0002

Specifications: 0.5ml

Storage conditions

4°C protect from light for 1 year; -20°C protect from light for 2 years

product description:

Polybrene is a polycationic polymer, often used in mammalian cell DNA transfection experiments to enhance the transfection efficiency of liposomes. Polybrene is currently widely used in retrovirus-mediated gene transfection and lentivirus-mediated gene transfection. The mechanism of action may be to neutralize the electrostatic repulsion between sialic acid and virus particles on the cell surface to promote adsorption. Polybrene is also a well-known anti-heparin agent (heparin antagonist), commonly used to produce non-specific agglutinated red blood cells. In addition, Polybrene is also mostly used for protein sequencing, because a small dose of Polybrene can significantly improve the degradation of peptides in automated sequencing analysis. The addition of polybrene to PVDF membrane can also increase the affinity of the membrane. This product is a ready-to-use solution. The powder is made into a 10mg/ml solution with 0.9% NaCl and filtered with a 0.22μM filter to sterilize. Generally, the dilution ratio is 1:1000-1:2000 when used. The dilution ratio varies according to the cell type. Please refer to relevant literature for details. Note: Polybrene is more toxic to certain cells (such as terminally differentiated neurons, DC cells), and it is recommended to do a toxicity test for the first application.

Kit components:

Name	Specification
Polybrene（hexadimethrine bromide） Concentration: 10ug/ul, dilution 1:1000-1: 2000	0.5mL

Instructions:

Experiment 1: Retroviral Infection (Retroviral Infection)

(1) Preparation of recombinant retrovirus stock solution: Take 5ml of growth medium (5% serum) into a 100mm culture dish containing a monolayer of transfected reverse transcription packaging cells. After 24 hours of incubation, the culture solution was aspirated and filtered with a 0.45 μm filter.

(2) Cultivation of cells to be infected: 10ml of complete medium is added to a 100mm culture plate, and the cell density is 5×105/plate.

(3) Virus infection: After the cells are cultured for 24 hours, aspirate the complete culture solution. Infect cells with 2ml virus supernatant containing polybrene (or dilute the virus stock solution to 2ml). The final concentration of polybrene is 5μg-10μg/ml. Incubate at 37° C for 3-6h.

(4) Collect virus particles: add 8ml complete medium. After 3 days of infection, cells were lysed with selection medium in a ratio of 1:5.

Experiment 2: Transfection

(1) Cultivate cells in a complete growth medium with a cell density of about 50%;

(2) Prepare the DNA-medium-Polybrene mixture after incubating the cells for 18-24 hours, and prepare the mixture as follows: ①Add complete medium (2ml for 60mm petri dish, 3ml for 100mm petri dish) and preheat at 37° C; ②Add Gently mix 10ng~10μg plasmid; ③Add Polybrene to a final concentration of 5μg-10μg/ml. Mix gently. Each of the above ingredients needs to be added in order.

(3) Remove the medium, add DNA-medium-Polybrene solution to the cells, and incubate the cells for 6-20h at 37° C. Mix gently every 1.5 hours during the first 6 hours of cell culture.
(4) Remove the DNA-medium-Polybrene solution. Cover the cells gently with DMSO shock solution (15% DMSO in 1X HBSS) (60mm culture
(5) Immediately remove the DMSO shock solution, and gently wash the cells twice with complete growth medium. For 60mm petri dishes, wash with 5ml culture fluid each time, and for 100mm petri dishes with 10ml culture fluid each time. 3ml dish, 4ml 100mm petri dish). Gently shake the culture plate by hand for 10 seconds each time the solution is added to make the liquid evenly distributed. Then incubate the cells at 37° C for 4 min.
(6) Add complete medium to the cells;
(7) Stable transformation: Remove the growth medium and lyse the cells with the selection medium in a ratio of 1:5. Transient expression: Remove the growth medium and add fresh growth medium. Harvest cells after 24-72h.

Use concentration (see literature for detailed steps):

The specific concentration of Polybrene depends on the cell type and transfection method. The following concentration is for reference only.
(1) In order to improve the transfection efficiency of adenovirus and LacZ transgene expression, 6ug/ml Polybrene was used to treat the viral vector and then transferred to BHK-21 cells (MOI was 100), the transfection rate was increased to 95%, and only those who were not treated with Polybrene 2.3% transfection rate.
(2) In the KSHV (Kaposi's sarcoma-associated herpesvirus) purification and transfection experiment, the concentrated virus and 8ug/ml Polybrene were added to BCBL-1 cells and incubated at 37°C for 2h.
(3) In the retrovirus construction study, keratinocytes (Keratinocytes) were transfected with v-rasHa retrovirus and cultured in a medium containing 4μg/ml Polybrene for 3 days, the virus titer was 1×107 virus/ml, MOI is 1[4].
(4) In the transfection study of shRNA-encoded lentiviral vector, 8μg/ml Polybrene was added to the viral supernatant and transfected into HEK293 cells [5].
(5) In the study of lentiviral shRNA transfection, a fresh medium containing 6μg/ml Polybrene was added to RCC10 cells cultured for 24 hours, and the cells were transfected with lentiviral particles.

It can only be used for scientific research. It is forbidden to use it for human, animal or other purposes.