HEPES Buffered Saline Solution (2× HeBS, pH 6.7)

Cat #: A-CSH419

Size: 100mL

Storage: -20°C (12 months)

Product Description

There are various methods for introducing exogenous genes into eukaryotic cells, such as calcium phosphate transfection, DEAE-dextran transfection, lipofection, electroporation, and microinjection. 2× HEPES Buffer Salt Solution is mainly used for calcium phosphate transfection. Its main component is HEPES, which is a zwitterionic buffering agent. It can effectively control the pH within the range of 6.8 to 8.2, especially exhibiting good buffering capacity at pH 7.2 to 7.4. The final concentration is generally 10 to 50 mmol/L, and a concentration of 20 mmol/L HEPES in the culture medium can achieve good buffering capacity.

HEPES Buffer Salt Solution (2× HeBS) is a commonly used cell transfection solution, mainly composed of HEPES, sodium chloride, and phosphate. The optimal pH value is 7.05 to 7.12. This product has a pH of 6.7 after sterile filtration. The factors that affect the efficiency of calcium phosphate transfection mainly include the DNA content in the precipitate, the duration of DNA staying on the cells, and the shock time. For 2× HeBS, the recommended DNA concentration is 10 to 50 μg, and cell lines such as Hela and BALB should be allowed to precipitate for 16 hours, while cell lines such as CHO, DUKX, and B Ⅱ can be subjected to heat shock treatment using glycerol or DMSO to improve transfection efficiency.

Usage

1. 24 hours before transfection, use trypsin digestion to culture cells. Transfer an appropriate number of cells to a new culture dish to ensure good cell growth during transfection.

2. 2 to 4 hours before adding DNA, add complete culture medium in a ratio of 9 mL/10 cm culture dish and incubate in a 37°C, 5% CO2 incubator.

3. Dissolve the DNA precipitated with ethanol in 450 μL of sterile water, add 50 μL of 2.5 M CaCl2, and mix thoroughly

to achieve a final Ca2+ concentration of 0.25 M.

4. Using a pipette, add 2× HeBS while slowly adding the prepared DNA/CaCl2 solution (the operation should be quick, generally within 30 to 60 seconds), vigorously shake for 5 seconds, and let it stand at room temperature for 20 to 30 minutes to form a precipitate.

5. Add the precipitate evenly to the cells in the culture dish, gently shake to mix the precipitate with the culture medium, and incubate in a 37°C, 5% CO2 incubator for 4 to 16 hours. If the cultured cells are CHO, DUKX, etc., shock treatment with glycerol or DMSO can greatly increase transfection efficiency. After 4 to 6 hours of culture, replace the current culture medium with 2 mL of complete culture medium containing 10% glycerol or 20% DMSO, let it stand at room temperature for 3 minutes, and shake with 5 mL of PBS.

6. Remove the culture medium, wash the cells twice with PBS, and add 5 to 10 mL of complete culture medium for further incubation.

7. For transient transfection, collect and detect cells at different time points after transfection, generally within 12 to 60 hours.

8. For stable transfection, incubate in non-selective culture medium for 18 to 48 hours after transfection, with a general duration of 24 to 36 hours to allow expression of the exogenous gene.

9. Digest the cells with trypsin and passage them, replace with appropriate selective culture medium for further incubation. Replace the selective culture medium every 2 to 4 days. Typically, the desired cell clones will appear within 10 to 14 days.

Notes

1. Pay attention to aseptic operation and try to avoid contamination.

2. For transient transfection, DNA precipitation with ethanol is not necessary.

3. Shock treatment of certain cell lines can greatly improve transfection efficiency, but be cautious as prolonged exposure to glycerol may cause cell death.

4. After 12 to 24 hours of transfection, the addition of 10 mmol/L sodium butyrate solution can increase viral titer.

5. The transfection efficiency of this reagent should be tested during transfection, and a good transfection efficiency should be between 30% and 60%.

6. For your safety and health, please wear lab coat and disposable gloves during the operation.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.