HEPES Buffered Saline Solution (2× HeBS, pH 7.05)

Cat #: A-CSH420

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, microinjection, etc. 2× HEPES Buffer Salt Solution is primarily used for calcium phosphate transfection. Its main component is HEPES, a zwitterionic buffering agent that effectively controls the pH in the range of 6.8 to 8.2, with optimal buffering capacity at pH 7.2 to 7.4. The final concentration of HEPES is generally 10 to 50 mmol/L, and a concentration of 20 mmol/L HEPES in the culture medium provides good buffering capacity.

HEPES Buffer Salt Solution (2× HeBS) is a commonly used cell transfection solution composed of HEPES, sodium chloride, phosphate, etc., with the optimal pH range of 7.05 to 7.12. It has been subjected to sterile filtration. Factors affecting the efficiency of calcium phosphate transfection include DNA content in the precipitate, the time DNA stays on cells, and the shock time. The recommended DNA concentration for 2× HeBS is 10 to 50 μg. Cell lines like Hela and BALB should be allowed to settle for 16 hours, while CHO, DUKX, B Ⅱ cells can be subjected to heat shock treatment using glycerol or DMSO to enhance transfection efficiency.

Usage

1. Digest the cultured cells with trypsin 24 hours before transfection. Transfer an appropriate number of cells to a new culture vessel to ensure their optimal growth during transfection.

2. Add complete culture medium in a ratio of 9 mL per 10 cm culture dish, 2 to 4 hours before adding DNA, 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 2.5M CaCl2 and mix thoroughly to

achieve a final Ca2+ concentration of 0.25M.

4. While pipetting 2× HeBS, add the prepared DNA/CaCl2 solution dropwise (the process should be quick, generally within 30 to 60 seconds) and vigorously vortex for 5 seconds. Allow the mixture to settle 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 swirl to ensure thorough mixing, and incubate at 37°C, 5% CO2 for 4 to 16 hours. For cell lines like CHO and DUKX, shock treatment with glycerol or DMSO can significantly increase transfection efficiency. After 4 to 6 hours of incubation, replace the current culture medium with 2 mL of complete culture medium containing 10% glycerol or 20% DMSO, let it sit at room temperature for 3 minutes, and then 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 analyze cells at different time points after transfection, generally within 12 to 60 hours.

8. For stable transfection, incubate the cells in non-selective culture medium for 18 to 48 hours after transfection, with the optimal time usually between 24 to 36 hours for exogenous gene expression.

9. Digest the cells with trypsin and passage them, replace with appropriate selective culture medium for further cultivation. Change the selective culture medium every 2 to 4 days, and typically, after 10 to 14 days, target cell clones will appear.

Notes

1. Pay attention to aseptic techniques and minimize contamination.

2. Ethanol precipitation of DNA is not necessary for transient transfection.

3. Shock treatment of certain cell lines can significantly increase transfection efficiency, but be cautious as prolonged exposure to glycerol can lead to cell death.

4. After 12 to 24 hours of transfection, the addition of sodium butyrate solution with a final concentration of 10 mmol/L can enhance virus titer.

5. The transfection efficiency of this reagent should be evaluated during transfection, with the desired efficiency ranging between 30% and 60%.

6. For your safety and well-being, wear appropriate lab attire and disposable gloves during the procedure.

Disclaimer

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