Calcium Phosphate Cell Transfection Kit

Product description

Calcium phosphate transfection is a common method for introducing DNA plasmid into eukaryotic cells. The principle is to mix phosphate buffer with $CaCl_2$ solution containing DNA plasmid to form DNA-calcium phosphate precipitate. This precipitate can adhere to the cell and eventually transfer DNA into the target cell by endocytosis.

This method has been successfully used in many cell lines to obtain transient or stable transfection. For example, HEK293 cell is one of the most suitable cells for calcium phosphate transfection. Other common cells (such as Hela and CHO cell) are also suitable for calcium phosphate transfection, but the transfection efficiency is slightly lower than that of HEK293 cell. This kit is modified on the basis of the traditional calcium phosphate method to achieve higher transfection efficiency and lower cytotoxicity, which is suitable for most transfection needs of adherent cell or suspended cell.

Composition and storage conditions

Components \ Size	100 T	200 T
2M $CaCl_2$	10 mL	20 mL
2X HBS formula	10 mL	20 mL
Store the components at 4 °C.		

Experimental operation

Taking the 6-well plate as an example, if the transfection vessel is different, please adjust the dosage proportionally.

I Adherent cell transfection

1. The cell is counted, seeded ((7-8)×10^5 cells each well is appropriate), and cultured in a 5% CO_2 incubator at 37°C for 18-24 h. Transfection is performed at 70-90% cell density.
2. 30-60 min before transfection, replace 2 mL fresh culture medium, and heat up the reagents required for transfection to 37°C.
3. Mix 16.5 μL 2M $CaCl_2$ solution and appropriate DNA plasmid with ddH_2O to prepare 125 μL DNA-$CaCl_2$ complex. Note that the amount of DNA plasmid is controlled at 1-5 μg per well.

4. 125 μL 2X HBS formula solution was added to the above DNA-$CaCl_2$ complex, mixing gently to form DNA-$CaCl_2$-HBS transfection solution.
5. Add the transfection solution evenly to each well. Gently shake the plate to distribute the transfection solution.
6. After 4-6 h culture, the medium is replaced by 2 mL fresh medium. At this point, obvious particle precipitation can be observed under the microscope.
7. Continue the culture for 24 h, the transfected gene expression was detectable usually.

II Suspension cell transfection

1. Collect suspension cell by low-speed centrifugation and wash once with PBS.
2. Same as steps 3 and 4 above, prepare DNA-$CaCl_2$-HBS transfection solution.
3. Resuspend each 10^6 cell pellets with 100 μL transfection solution for 20-30 min at room temperature.
8. Add 2 mL fresh medium per well of the 6-well plate, and the above cell transfection suspension was evenly seeded into the well. Gently shake the plate to distribute the transfection solution.
4. After 4-6 h of culture, centrifuge the the cells to remove the medium, and then wash once with PBS, replaced with 2 mL fresh medium.
5. Continuing the culture for 24 h, the transfected gene expression should be detectable usually.

Notes

1. High purity plasmid is necessary for high transfection efficiency, ensuring A260/A280 > 1.8, and electrophoresis is recommended for plasmid integrity.
2. Although shock treatment for some cell lines can greatly improve transfection efficiency, it should be noted that excessive glycerol exposure can easily lead to cell death.
3. The pH value of HBS formula is related to the transfection efficiency. Avoid CO_2 acidification of HBS formula from prolonged exposure to air.
4. Prepare sufficient transfection solution to reduce the variation during transfection for multiple well plates.
5. Aseptic operation to avoid contamination, and the DNA plasmid should not contain proteins and phenols.
6. This product is for scientific use only.

Troubleshooting

1. Low transfection efficiency

Probable Causes	Corresponding Suggestion
Poor form of calcium phosphate-DNA precipitation	Adding 2X HBS solution too fast or too little will result in poor precipitation. Mixing the 2X HBS and DNA-$CaCl_2$ should be gently and continuously.
Poor DNA quality	The A260/A280 ratio of DNA plasmid should ≥ 1.7.

pH is not optimal	The pH of the HBS formula should be between 7.05-7.12. Prolonged storage may cause changes in the pH of the formula. Therefore, it should be used as soon as possible after purchasing the kit.

2. Transfection efficiency is inconsistent

Probable Causes	**Corresponding Suggestion**
Inconsistency of cell density	Keep cell density as constant as possible. Transfection is usually performed at the cell density of 70%-90%.
Poor cell growth	Maintain healthy cell growth. Cell should be transfected in the middle of the log phase growing. Excessive passage of cell lines will reduce transfection efficiency.

3. Toxicity

Probable Causes	**Corresponding Suggestion**
Cell death when calcium phosphate addition or within 24 h	Optimize cell density, higher cell density helps maintaining cell survival.
	Optimize the incubation time of cells and transfection reagents. Certain cells may be more sensitive to calcium phosphate.
	Transfected gene products could be toxic.