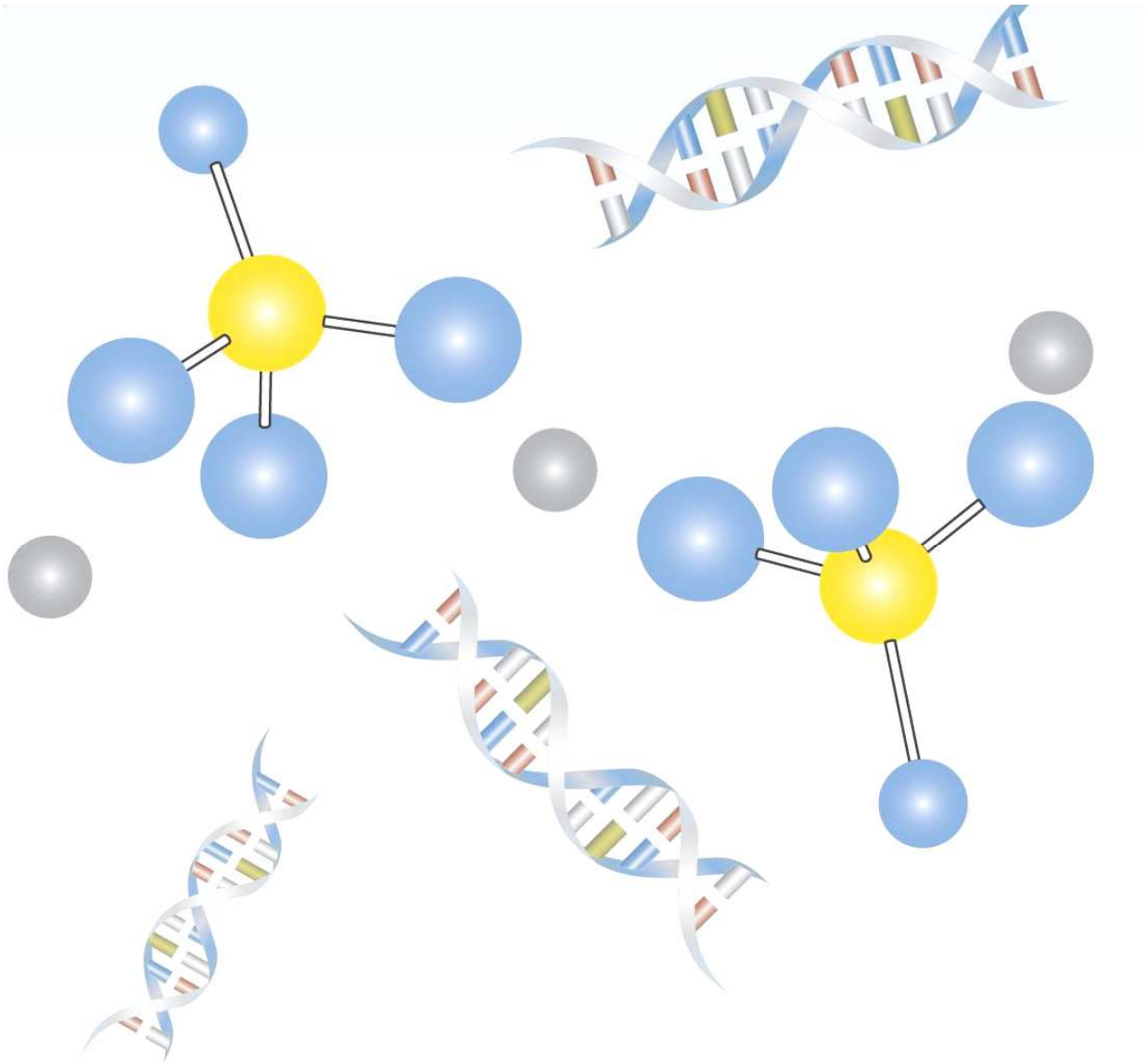
Calcium Phosphate Transfection Kit

INSTRUCTION MANUAL



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Calcium Phosphate Transfection Kit

Instruction Manual

Calcium Phosphate Transfection Kit
Perfect for virus production in HEK 293 cells

Kit Contents

Catalog Number	Description	Volume (mL)	Size (number of transfection / 100 mm dish)*
CP90000	- 1X Hepes Buffered Saline (HBS) - 2.5 M $CaCl_2$	4 x 15 mL 3.5 mL	100

* Calcium Phosphate Transfection Kit allows performing 500 transfections in 6 well plates or 35 mm dishes.

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

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1. Technology

1.1. Description

Congratulations on your purchase of the Calcium Phosphate Transfection Kit!
The calcium phosphate transfection method was first described by Graham and Van der Ebb in 1973. This method was adapted by several other teams in order to reach higher transfection efficiencies. Calcium phosphate forms a fine precipitate by interacting with DNA allowing the formation of small complexes which are internalized into mammalian cells and transfect them. Although this procedure can be routinely used to transfect a wide variety of cell lines, it gives superior results with the very popular HEK 293 cell line. However, laborious optimizations are usually required to obtain the best of this method. We have modified and optimized this technique to provide you a very reproducible and efficient Calcium Phosphate Transfection Kit. It will allow reaching between 95% and 100 % HEK 293 transfected cells in your routine experiments.

Principal Calcium Phosphate Transfection Kit advantages:

1. Compaction of DNA in nanoparticles efficiently internalized by cells.
2. Protection of nucleic acids against nucleases degradation.
3. Modified and optimized to reach higher transfection level.
4. Ready to use.

1.2. Stability and Storage

Storage: – 20°C. Upon receipt and for long-term use, store all reagent tubes at – 20°C. The Calcium Phosphate Transfection Kit is stable for at least one year at the recommended storage temperature.

Shipping condition: + 4°C.

2. Applications

The calcium phosphate transfection method can be used to transfect DNA in many cell types. It is particularly efficient to transfect HEK 293 cells and produce retroviruses or lentiviruses for cell transduction applications. It can also be used to easily produce recombinant proteins. The Calcium Phosphate Transfection Kit is compatible with serum-containing culture media. This product is ready-to-use and intended for research purpose only.

3. General Protocols

3.1. General Considerations

The instructions given below represent the optimized protocol that was applied successfully in our R&D facility. However, optimal conditions may vary depending on the nucleic acid quality, cell types and size of cell culture dishes. Therefore, the amounts of DNA may have to be adjusted to achieve best results.

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used.
- **Nucleic acids** should be as pure as possible. Endotoxins level must be very low since they interfere with transfection efficiencies. Moreover, we suggest avoiding long incubation time of the DNA solution in HBS buffer before the addition of the $CaCl_2$ solution to circumvent any degradation or surface adsorption.

- **Antibiotics**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the Calcium Phosphate Transfection Kit.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the DNA calcium phosphate precipitates.

3.2. Cells Preparation

Adherent cells. It is recommended to seed or plate the cells in Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % of fetal bovine serum the day prior transfection. The use of RPMI culture medium during the transfection must be avoided. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should be between 50% and 80% confluent at the time of transfection (see the suggested cell number in the Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Table 1: Cell number, DNA amount and volume of buffers.

Tissue Culture Dish	Cell Number	DNA Quantity (µg)	HBS 1X Volume (µL)	$CaCl_2$ Volume (µL)	Culture Medium Volume
96 well	$0.05 – 0.2 \times 10^5$	0.25	15	0.8	100 µL
24 well	$0.5 – 1 \times 10^5$	1	30	1.7	500 µL
12 well	$1 – 2 \times 10^5$	2	60	3.3	1 mL
6 well	$2 – 5 \times 10^5$	4	120	6.6	2 mL
60 mm dish	$5 – 10 \times 10^5$	8	240	13.2	4 mL
90 - 100 mm	$10 – 30 \times 10^5$	20	600	33	8 mL
T-75 flask	$20 – 50 \times 10^5$	25	750	41	10 -12 mL

Stable transfection. The same protocol can be used to produce stably transfected cells except that 48 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait for at least 48 hours before exposing the transfected cells to selection media.

3.3. Protocol

The DNA and all Calcium Phosphate Transfection Kit solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is given to transfect cells in a 100 mm dish. For other types of vessel you can follow the amount and volumes indicated in the Table 1 or use the protocol described below and then add the appropriate volume of DNA complexes (DNA amount) to the cells. It is important to maintain the ratios of DNA, 1X HBS and $CaCl_2$ specified in the Table 1.

1) **Cell preparation**. Plate the cells at the required density in DMEM containing 10 % FBS and incubate overnight at 37°C in a CO_2 incubator. DO NOT use RPMI culture medium.

2) **Medium change**. Replace the cell culture medium by fresh culture medium 1-2 h before the transfection. DO NOT use old culture medium. It is important to only use well-pH culture medium.

3) **DNA solution**. Dilute 20 µg of DNA in 600 µL (see Table 1) of 1X HBS. If several plasmids need to be transfected (virus production), use the same amount of each plasmid up to 20 µg total DNA quantity.

4) **Calcium phosphate / DNA precipitation**. Add 33 µL of $CaCl_2$ solution and mix immediately by brief vortexing. Incubate the mixture at room temperature for 30 minutes.

5) **Transfection**. Add the complexes drop by drop to the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

6) **Cell incubation**. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to 72 hours following transfection.

Comments:

- For some cells, 24 hours post-transfection, replace the old media with fresh media.
- Keep unchanged the DNA amount, and respective volumes of 1X HBS and $CaCl_2$ detailed in Table 1.
- If you transfect small DNA quantities, you can adapt the protocol as follow. For example, to transfect 1 µg of DNA / well in a 24-well plate, use 30 µL of 1X HBS and 1.7 µL of $CaCl_2$ as indicated in Table 1. Proceed as described above until the step 4). After the 30 min of incubation, you can add some DMEM without serum (such as 70 µL) to the Calcium Phosphate / DNA mixture in order to get a sufficient volume to disperse the complexes drop by drop on cells.

3.4. Example of Virus Collection Procedure

1) Day 1: Perform the transfection.
2) Day 2 at 8 am: Replace the tissue culture medium (10 mL / 100 mm dish).
3) Day 2 at 2 pm: Remove the tissue culture medium and add 5 mL of fresh medium.
4) Day 2 at 8 pm: Harvest virus. Collect in a tube 3 mL of medium and replace by 3 mL of fresh medium. Keep the virus supernatant on ice at all times during the harvesting procedure and store it at 4°C.
5) Day 3 at 8 am: Repeat the step 4.
6) Day 3 at 2 pm: Repeat the step 4.
7) Day 3 at 8 pm: Repeat the step 4.
8) Day 4 at 8 am: Collect the 5 mL of medium.
9) Pool all virus supernatants harvested (about 17 mL).
10) Filter with 0.45 µm nitrocellulose filter.
11) Freeze in small aliquots and store at –70°C.
12) Determine the titer of your virus supernatant after one cycle of freezing / thawing.

Note: Do not perform multiple cycles of freezing/thawing since the virus supernatant titer is reduced dramatically each time.

4. Appendix

4.1 Quality Controls

To insure the performance of each lot of the Calcium Phosphate Transfection Kit produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Transfection efficacies on HEK 293 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

4.2. Troubleshooting

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. You can contact them at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com for updated information.

Problems	Comments and Suggestions
Low transfection efficiency	1- **Poor quality of DNA.** Nucleic acids should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies. Employ nuclease-free materials. We recommend the use of Endotoxin-free plasmid DNA preparation kit. 2- **DNA concentration.** The DNA concentration should not be less than 0.5 mg / mL. We recommend the use of 0.5X HBS or water as dilution buffers when DNA is intended to be used with the Calcium Phosphate Transfection Kit. 3- **pH not optimal.** a) pH of HBS may change during prolonged storage. Make sure you used the transfection kit within the indicated shelf life. b) pH of culture medium. Perform a medium change 1-2h before the transfection. Do not use old DMEM culture medium (pink color) meaning that its pH is not optimum. Do not buffered DMEM with Hepes solution. 4- **Poor calcium phosphate / DNA precipitate formation.** Follow exactly the described protocol and keep constant all the indicated volumes of 1X HBS, $CaCl_2$ solutions as well as the DNA quantity. 5- **Presence of large aggregates.** Do not perform the transfection in RPMI culture medium. 6- **Cell condition.** Cells should be healthy and in their growing phase the day of transfection. The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. Note also that transfection efficiency can decrease for cells that have been cultured too long. 7- **Old transfection reagent / DNA complexes.** The calcium phosphate / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hour can be aggregated. 8- **Perform complexes at room temperature.** All components of the Calcium Phosphate Transfection Kit must be warmed at room temperature before use. 9- **Addition of complexes to the cells.** Add the complexes drop by drop to the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g X-Gal powder 1g / G-418, Sulfate 1g

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

Purchaser Notification

Limited License

The purchase of the **Calcium Phosphate Transfection Kit** grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed herein). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

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