



Transfection reagent

Calcium Phosphate Transfection Kit

HEK 293 cells transfection
Virus Production

Protocol

Calcium Phosphate Transfection kit | Specifications

Package content	CP90000: 4 x 15mL of 1x Hebes Buffer Saline (HBS) + 3.5mL of 2.5M CaCl ₂
Shipping conditions	Gel Pack.
Storage conditions	Upon reception, store the Calcium Phosphate transfection kit at -20°C.
Shelf life	1 year from the date of purchase when properly stored and handled.
Product description	The CaPO transfection kit is simple and easy to use. It allows reaching between 95 and 100% of HEK 293 transfected cells and a very high titer for virus production.
Important notice	For research use only. Not for use in diagnostic procedures.

For additional information and protocols, tips, troubleshooting or other applications:

 www.ozbiosciences.com

Any questions?

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Protocol | For DNA transfection

1. Cells preparation

- It is recommended to plate the cells in Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % of fetal bovine serum, or Opti-MEM 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.

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 x 10 ⁵	0.25	15	0.8	100 µL
24 well	0.5 – 1 x 10 ⁵	1	30	1.7	500 µL
12 well	1 – 2 x 10 ⁵	2	60	3.3	1 mL
6 well	2 – 5 x 10 ⁵	4	120	6.6	2 mL
60 mm dish	5 – 10 x 10 ⁵	8	240	13.2	4 mL
90 - 100 mm	10 – 30 x 10 ⁵	20	600	33	8 mL
T-75 flask	20 – 50 x 10 ⁵	25	750	41	10 -12 mL

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

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. Reagent preparation

- a. *DNA solution*. Dilute 0.25 to 25 µg of DNA in 15 to 750 µL of HBS (see Table 1).
- b. *Calcium Phosphate / DNA Precipitation*: Add 0.8 to 41 µL of CaCl₂ solution and mix immediately by brief vortexing.
- c. Incubate the mixture at room temperature for 30 min.

IMPORTANT NOTE:

- It is important to maintain the ratios of DNA, 1X HBS and CaCl₂ specified in the Table 1

4. Transfection.

- a. Add the complexes in a dropwise manner onto the cells growing in complete culture medium and homogenize by rocking the plate back and forth to ensure a uniform distribution of the mixture.
- b. Incubate the cells at 37°C in a CO₂ incubator under standard conditions until evaluation of the protein expression. Depending on the cell type and promoter activity, the assay can be performed 24 to 72h following transfection.

Note: For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.

Protocol | For co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. For example, if you have two DNA plasmids, mix 0.5 µg of each plasmid, precipitate the 1 µg of DNA in 30 µL 1X HBS with 1.7 µL of CaCl₂.

Protocol | For 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.

Additional products:

- **DreamFect Gold** for transfection of all types of nucleic acid.
- **Polymag Neo** a magnetic beads-based transfection reagent dedicated to hard-to-transfect cells.

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 in this protocol). 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. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the Calcium Phosphate Transfection kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Calcium Phosphate Transfection kit reagents and documentation to OZ Biosciences, or by destroying all Calcium Phosphate Transfection components. Purchasers are advised to contact OZ Biosciences with the notification that a Calcium Phosphate Transfection kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the Calcium Phosphate Transfection kit research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

Calcium Phosphate Transfection kit and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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