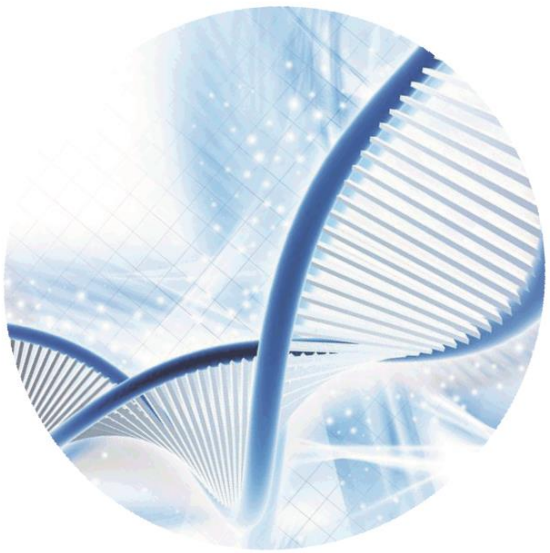


Transfection reagent

VeroFect

The solution for Vero Cells

Protocol

OZBIOSCIENCES
The art of delivery systems

VeroFect™ Quick Protocol

To find the ideal conditions, VeroFect must be tested at ratios **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of VeroFect / µg of DNA). For the DNA quantity, we suggest **0.25 µg** per well in 96-well, **0.5 µg** per well in 24-well and **2 µg** per well in 6-well.

1

Seed cells to be at 70% confluent the day of transfection

2

Prepare 3 identical tubes of DNA

96 well plate	24 well plate	6 well plate
0.5µg in 25µL of serum-free medium or buffer x 3	1µg in 50µL of serum-free medium or buffer x 3	4µg in 100µL of serum-free medium or buffer x 3

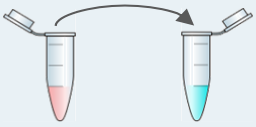
3

Prepare 3 tubes of VeroFect (with 3 different amounts of reagent)

96 well plate	24 well plate	6 well plate
0.25µL/0.5µL/0.75µL in 25µL of serum-free medium or buffer	0.5µL/1µL/1.5µL in 50µL of serum-free medium or buffer	2µL/4µL/6µL in 100µL of serum-free medium or buffer

4

Mix each tube of DNA (step 2) to each tube of VeroFect (step 3)

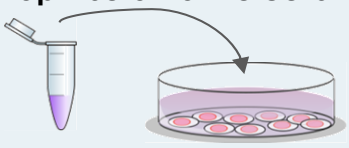


5

Incubate 20 min at room temperature

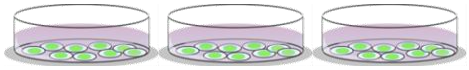
6

Distribute each mix dropwise onto the cells to insure uniform distribution



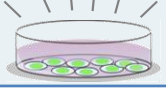
7

Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression



8

Choose the best ratio DNA:VeroFect



IMPORTANT NOTES – Before you begin

- ✓ The conditions provided above might require some further optimizations depending on your cells, DNA, RNA, etc…
- ✓ For Vero cells, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ During preparation of complexes, prevent VeroFect reagent solution to come into contact with any plastic surface that could result in material lost by adsorption. First, add serum-free culture medium to the tube and then mix VeroFect directly into the solution.
- ✓ Medium or buffer without serum & supplement must be used for the DNA/VeroFect complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ Dilute the reagent with deionized water for doses less than 1µL.
- ✓ For some cells, 24h post-transfection replace the old medium with fresh pre-warm media or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be replaced after 3-4h.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	VF60250: 250 µL of VeroFect VF60500: 500 µL of VeroFect VF61000: 1 mL of VeroFect VF65000: 5 x 1 mL of VeroFect
Shipping conditions	Room Temperature
Storage conditions	Store the VeroFect transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	VeroFect is a formulation specifically designed to obtain highly efficient and reproducible transfection of Vero cells.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

It is recommended to seed or plate the cells the day prior transfection. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of transfection. The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval (> 48h), a lower density should be used.

Tissue Culture Dish	Cell Number
96 well	0.08×10^5
24 well	0.5×10^5
6 well	2×10^5

Table 1: Cell number suggested

2. DNA/VeroFect complexes preparation

a. *VeroFect*: Vortex the reagent and dilute the indicated quantity of VeroFect in 25 to 100 µL of culture medium without serum and supplement (refer to table 2).

b. *DNA*: Dilute the indicated quantity of DNA (see Table 2) in 25 to 100 µL of culture medium without serum and supplement.

c. Add DNA solution to VeroFect solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 15-20min. Do not vortex or centrifuge.

3. Transfection

d. Add the complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

e. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression.

Note: in case of cells very sensitive to transfection, the medium can be changed after 24 hours incubation with fresh medium.

Tissue Culture Dish	DNA Quantity (µg)	VeroFect Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.25	0.5	2 x 25	200 µL
24 well	0.5	1	2 x 50	500 µL
6 well	2	4	2 x 100	2 mL

Table 2: Recommended DNA amount, VeroFect volume and transfection conditions

Protocol | Steps 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 0.25 µg of each plasmid, complex the 0.5 µg of DNA with 1 µL of VeroFect.

Option for Co-transfection.
Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Protocol | Steps for optimization

Due to the variability of DNA, cells and culture conditions, it is complex to provide optimal guidelines. In this context, it might be required to accomplish few optimizations to achieve the best results.

1. Quantity of DNA:

In order to obtain the highest transfection efficiency, the amount of DNA used can be optimized (as detailed in table 3), especially with plasmids having a weak promoter or with large DNA vector such as BAC vectors or virus encoding vectors. These effects vary with the number of cells so, it is important to always keep the number of cells and the incubation time constant during your optimization procedure.

Tissue Culture Dish	DNA Quantity (µg)	VeroFect Volume (µL)
96 well	0.2 – 0.8	0.4 – 1.6
24 well	0.5 - 3	1 - 6
6 well	3 - 8	6 - 16

Table 3: DNA and VeroFect range for optimization.

2. Quantity of VeroFect:

After optimization of DNA amount, the ratio VeroFect / DNA can be optimized by varying the amount of VeroFect (see table 3) while maintaining the quantity of DNA constant. For instance, with 1µg of DNA, used 1, 2, 3 µL of VeroFect.

3. Cell number:

For stable transfection, cells can be seeded with lower density and, taking into account the efficiency of VeroFect, the quantity of DNA used can be reduced. 48 to 72h post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48h before exposing the transduced cells to selection media.

4. Incubation time:

The optimal time range between transfection and assay for gene activity varies with promoter activity, expression product, etc. The transfection efficiency can be monitored after 24 - 72h.

Additional products

- **Helix-IN™** – Broad Spectrum DNA transfection reagent
- **PolyMag Neo** – magnetic beads-based transfection reagent dedicated to hard-to-transfect cells
- **pVectOZ Transfection plasmids (CAT, GFP, LacZ, Luciferase, SEAP)** - Positive controls and optimization of all transfection experiments

Purchaser Notification

Limited License
The purchase of the VeroFect 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 VeroFect 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 VeroFect kit reagents and documentation to OZ Biosciences, or by destroying all VeroFect components. Purchasers are advised to contact OZ Biosciences with the notification that a Verofect 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 VeroFect 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
VeroFect 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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