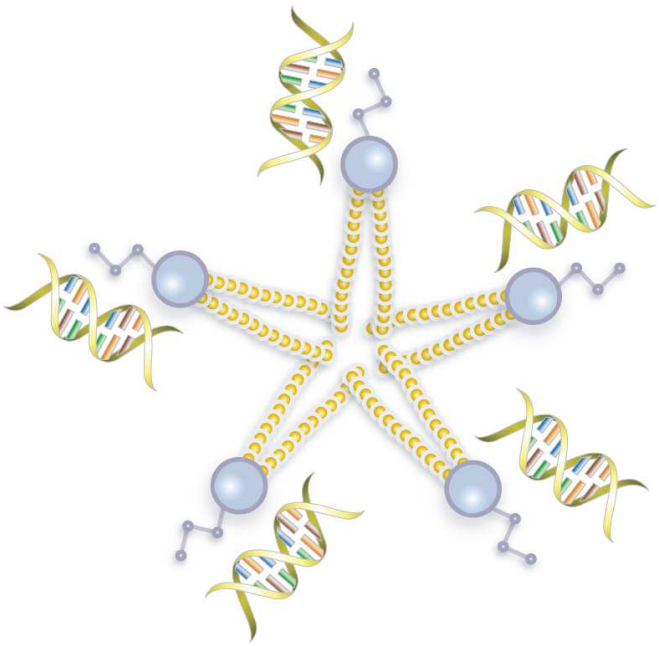
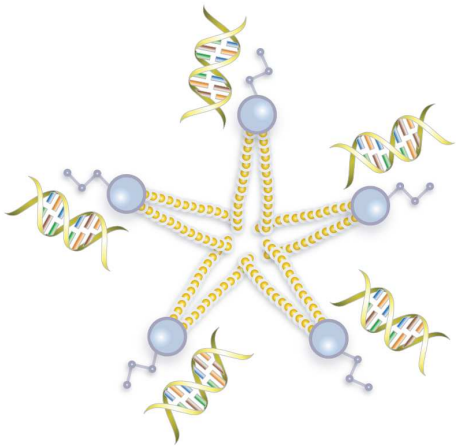
VeroFect

INSTRUCTION MANUAL





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VeroFect

Instruction Manual

VeroFect
The Vero cells specific transfection reagent

List of VeroFect Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
VF60250	VeroFect	250	125
VF60500	VeroFect	500	250
VF61000	VeroFect	1000	500
VF65000	VeroFect	5 X 1000	2500

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 ***VeroFect*** transfection reagent!
VeroFect is a specific reagent dedicated to the transfection of Vero cell lines (African Green Monkey kidney epithelial cells). VeroFect is based on our **Tee-Technology ("Triggered Endosomal Escape")**. The Tee-Technology combines and exploits the properties of cationic lipids and polymers to achieve an extremely efficient DNA delivery into cells. The association of nucleic acids with the VeroFect reagent results in the tight compaction and protection of the DNA. Then, these positively charged complexes bind to cell surface and are taken up by endocytosis. Inside the endosomes, the hydrophobic property of lipids acts in synergy with endosomes buffering capacity of the polycationic residues allowing: 1) a very efficient destabilization of the endosomal membrane 2) the release of large DNA amounts in the cytosol and 3) the DNA nuclear uptake.

Principal VeroFect advantages:

1. Compaction of DNA in nanoparticles efficiently internalized by cells
2. Protection of nucleic acids against nucleases degradation
3. Efficient membrane destabilization and DNA delivery into cells
4. Highly efficient and reliable
5. Simple, ready-to-use & rapid
6. Compatible with and without serum-containing culture media
7. Non toxic & economical

1.2. Kit Contents

OZ Biosciences offers four sizes of ***VeroFect*** reagent.

- One tube containing 250 µL of VeroFect good for 125 transfections with 1 µg of DNA
- One tube containing 500 µL of VeroFect good for 250 transfections with 1 µg of DNA
- One tube containing 1 mL of VeroFect good for 500 transfections with 1 µg of DNA
- 5 tubes containing 1 mL of VeroFect good for 2500 transfections with 1 µg of DNA

Stability and Storage
Storage: +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge (+4°C). VeroFect kits are stable for at least six months at the recommended storage temperature.

- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF VeroFect REAGENT!**

Shipping condition: Room Temperature.

2. Applications

VeroFect has been developed for very efficient transient and stable transfections of Vero cell lines. It is also efficient for other cell types. The VeroFect is compatible with serum-containing culture media and serum free culture media. This product is stable, ready-to-use and intended for research purpose only.

3. General Protocols

3.1. General Considerations

The instructions given below were specifically optimized to transfect Vero cells.

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency.
- **Nucleic acids** (plasmid) should be as pure as possible. Endotoxin levels must be very low since they hamper with transfection efficiencies. We suggest avoiding long incubation time of the DNA solution and VeroFect reagent in buffers or serum free medium before mixing to avoid 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 VeroFect reagent.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the DNA and transfection reagent solutions.

3.2. 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.

Table 1: Cell number

Tissue Culture Dish	Cell Number
96 well	0.08×10^5
24 well	0.5×10^5
12 well	1×10^5
6 well	2×10^5
60 mm dish	4.5×10^5
90 - 100 mm	12×10^5
T-75 flask	15×10^5

VeroFect reagent can also achieved outstanding results on other cell types. Please, do not hesitate to contact us about list of cells successfully tested with VeroFect.

3.3. Protocol

The DNA and VeroFect solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 2 µL of VeroFect per µg of DNA.

1) **DNA solution**. Dilute 0.4 to 20 µg of DNA in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics.
   VeroFect solution. Dilute 0.8 to 40 µl of VeroFect in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics. It is very important to add first the serum free medium to the tube and then add carefully the VeroFect reagent directly into the serum free medium without touching any plastic surface.
   - **Do not use serum-containing media for this step!**

- **Prevent the VeroFect and DNA stock solutions to come into contact with any plastic surface. First, add serum-free culture medium to the tube and then drop the VeroFect and DNA stock solution directly into the medium. Contact of VeroFect and DNA with the tube surface (plastic or glass) will result in materials lost by adsorption.**

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

Tissue Culture Dish	DNA Quantity (µg)	VeroFect Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.5	1	2 x 25	150 µL
24 well	1	2	2 x 50	500 µL
12 well	2	4	2 x 50	1 mL
6 well	4	8	2 x 100	2 mL
60 mm dish	8	16	2 x 150	4 mL
90 - 100 mm	20	40	2 x 250	8 mL
T-75 flask	20	40	2 x 350	10 -12 mL

2) Combine the two solutions, mix gently by carefully pipetting up and down and incubate the mixture for 20 minutes at room temperature.

- **The diluted solutions should be combined within 5 minutes.**
- **Do not vortex or centrifuge!**

3) Add the complexes 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.

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

- **Optionally, a medium change can be performed 24 hours post-transfection.**

3.4. Optimization Protocol

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.

Table 3: DNA and VeroFect range for optimization.

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
12 well	1 - 4	2 - 8
6 well	3 - 8	6 - 16
60 mm dish	6 - 10	12 - 20
T-75 flask	10 - 30	20 - 60

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 72 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours 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 - 72 hours.

4. Appendix

4.1 Quality Controls

Each lot of VeroFect produced is qualified 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
Purity	Silica Gel TLC assays. Every compound shall have a single spot.
Sterility	Thioglycolate assay. Absence of contamination shall be obtained for 7 days.
Biological Activity	Transfection efficacies on Vero cells. Every lot shall have an acceptance specification of > 85% 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. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

5. Related Products

Please visit our website (www.ozbiosciences.com) for information and details about OZ Biosciences other related products such as:

1) **Magnetofection™**: Super Magnetic Plate, 96-magnets Magnetic Plate, PolyMag, CombiMag, SilenceMag, ViroMag, ViroMag R/L, NeuroMag, FluoMag, SelfMag amino and SelfMag carboxy kit.
2) **TEE-Technology**: DreamFect™, DreamFect™ Gold, Lullaby siRNA™, EcoTransfect & FlyFectin™
3) **Protein Delivery Systems**: Ab-DeliverIN™ and Pro-DeliverIN™
4) **Other products:** GeneBlaster™, Bradford–PAK, -Galactosidase (ONPG) assay kits, -Galactosidase (CPRG) assay kits, X-Gal staining kit, DNA markers, shRNA GFP (pure), shRNA Luciferase (pure)...

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 ***VeroFect*** Reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). 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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Product Use Limitations

The VeroFect Reagent 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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