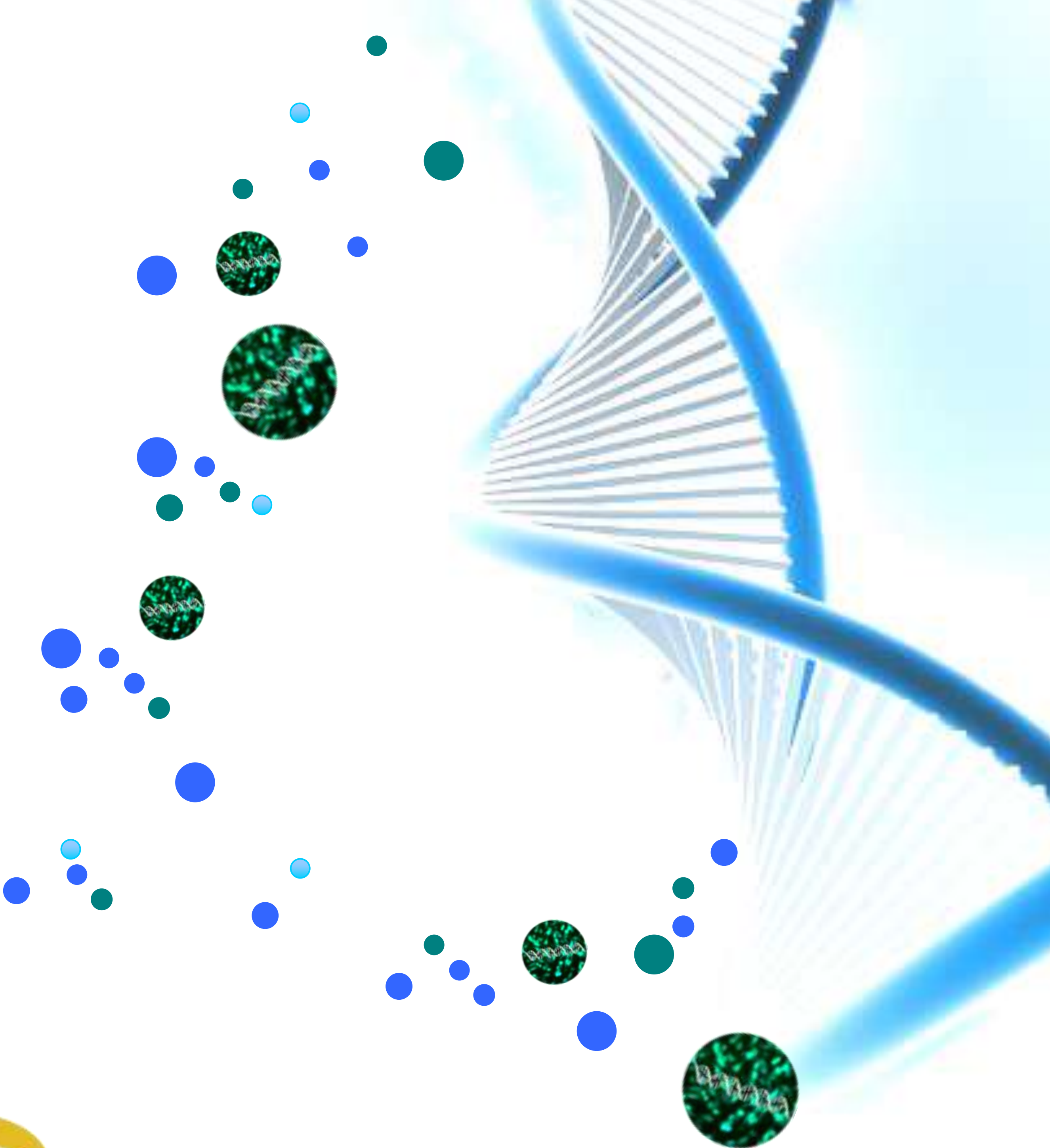
HeLaFect Transfection Reagent

INSTRUCTION MANUAL



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HeLaFect

Instruction Manual

HeLaFect is the newest transfection reagent specific for **HeLa cells** transfection.

List of HeLaFect kits

Catalog Number	Description	Volume (µL)	Number of transfections with 1 µg of DNA
HF20500	HeLaFect transfection reagent	500 µL	250
HF21000	HeLaFect transfection reagent	1 mL	500
HF25000	HeLaFect transfection reagent	5 x 1 mL	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 (order@ozbiosciences.com). 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

HeLaFect is the ideal transfection reagent specifically developed for HeLa cells transfection with high efficiency. **HeLaFect** is a lipid-based reagent based on the Tee-Technology ("Triggered Endosomal Escape"). The cationic design of **HeLaFect** reagent allows high nucleic acid compaction for an efficient transport directly into HeLa cells. This reagent is composed by biodegradable lipids leading to high viability and is ready-to-use.

HeLaFect transfection reagent principal advantages:

- Highly efficient with HeLa cells
- Ready-to-use: no need of additional buffer
- Low nucleic acid amount - minimized toxicity
- High level of nucleic acid compaction
- Easy and straightforward protocol
- Compatible with any culture medium: medium change not required

1.2. Kit Contents, Stability and Storage

Contents

Kits content varies according to their size:

- 1 tube containing 500 µL of HeLaFect reagent good for up to 250 assays with 1 µg DNA.
- 1 tube containing 1 mL of HeLaFect reagent good for up to 500 assays with 1 µg DNA..
- 5 x 1 tubes containing 1 mL of HeLaFect reagent good for up to 2500 assays with 1 µg mRNA.

Stability, Storage and Shipping

Stability: HeLaFect reagent and pVectOZ-GFP are stable for at least 18 months at the recommended storage temperature.

Storage: Upon reception and for long-term use, store the HeLaFect transfection reagent at -20°C.

Shipping condition: Room Temperature

HeLaFect transfection reagent is stable for several days at room temperature or at +4°C without losing activity. The storage at -20°C minimizes the size of liposomes and thus leads to higher efficiency. The numbers of freeze and thaw cycles do not affect the efficiency of the reagent.

2. Applications

HeLaFect has been developed specifically for nucleic acids transfection into HeLa lineages. This transfection reagent is serum compatible and is used for transient as well as stable transfection. This product is very stable, ready-to-use and intended for research purpose only.

3. General Protocol

3.1. General Considerations / Important Guidelines

The instructions given below represent standard protocol that was applied successfully with HeLa cells. Optimal conditions may vary depending on the plasmid, clone, size of cell culture dishes and conditions of culture. As a starting point, use **2 µL of HeLaFect per µg of DNA.** Refer to the optimization procedure to find optimal transfection conditions.

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. Use regularly passaged HeLa cells at confluence between 60 and 80% (visual confluence). Do not use cells that have been cultured for too long (> 2 months).

- **Nucleic Acids** should be as pure as possible and free of contaminants. We suggest avoiding long storage of the diluted nucleic acid solution before the addition of HeLaFect to circumvent any degradation or surface adsorption. We recommend using pVectOZ-GFP plasmid for an efficient transfection control.

- **Culture Medium**. 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 HeLaFect transfection reagent.

3.2. Cells Preparation

Cell culture prior to transfection: one day before transfection prepare the cells according to the table below.

It is recommended to plate the cells the day prior transfection in classical culture medium. Cells should be 60-80 % 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 protein expression 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 suggested (per well).

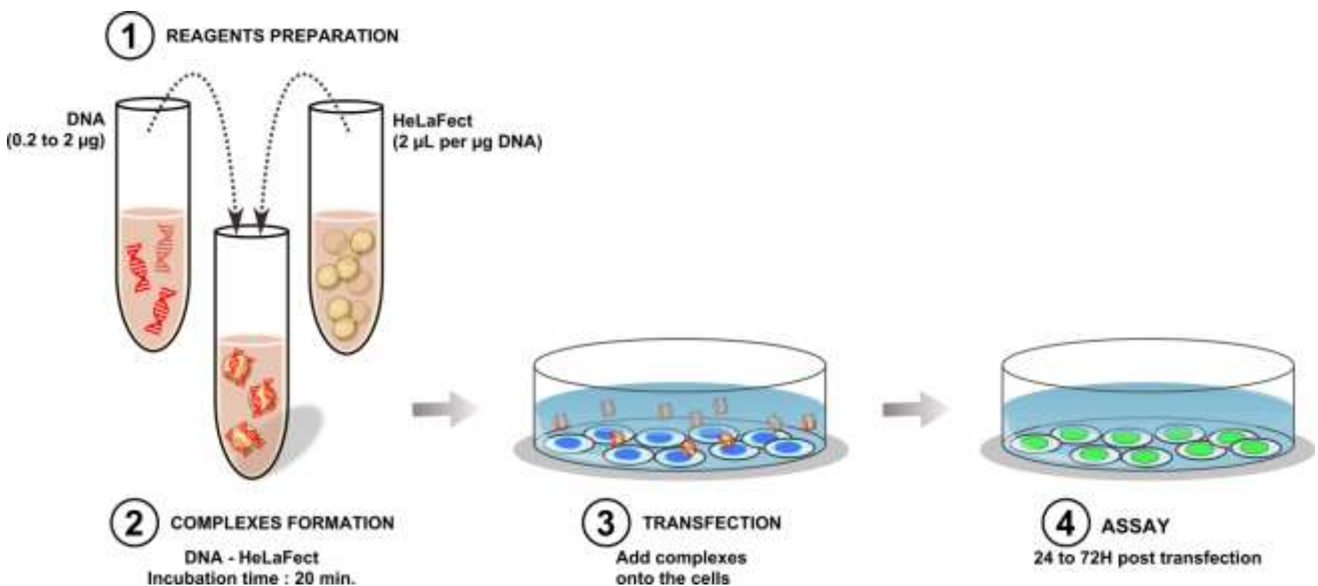
Tissue Culture Dish format	Surface area per well[1]	Cell Number
96 wells	0.3 cm^2	0.5 – 1.6 x 1.10^4
24 wells	2 cm^2	0.5 – 1 x 1.10^5
6 wells	10 cm^2	2 – 5 x 1.10^5

[1] Surfaces area may vary depending on the manufacturer.

3.3. DNA Transfection Protocol

Use the following procedure to transfect DNA into HeLa cells. The Table 2 shows optimized transfection conditions according to different cell culture formats (all amounts are given on a per-well basis).
For most HeLa clones: use **2 µL of HeLaFect per µg of DNA**

Note: We suggest beginning with the recommended ratios and optimize it, if required.



1) **Reagents preparation.** Allow reagents to reach room temperature before beginning.
   a. *DNA solution.* Dilute **0.2** to **2 µg** of DNA in **25** to **250 µL** of culture medium without any supplement (SVF, antibiotics, growth factors…) or PBS (refer to table 2).

   b. *HeLaFect solution.* Mix the reagent gently before use. Dilute **0.4** to **4 µL** of HeLaFect in **25** to **250 µL** of culture medium without any supplement (SVF, antibiotics, growth factors…) or PBS (refer to table 2).

Table 2: mRNA amount, HeLaFect volume and transfection conditions suggested (per well).

Tissue Culture Dish format	DNA Quantity (µg)	HeLaFect Volume (µL)	Dilution Volume (µL)[1]	Total culture medium Volume
96 wells	0.2	0.4	2 x 25	150 µL
24 wells	0.5	1.0	2 x 50	400 µL
6 wells	2.0	4.0	2 x 250	2 mL

[1] Volumes of dilution medium for steps 1a and 1b.

2) **Complexes formation.** Combine the DNA and the HeLaFect solutions. Mix gently by carefully pipetting up and down and incubate the mixture for 20 minutes at room temperature. Do not vortex or centrifuge!

   Note: Proceed to step 3 within 30 minutes

3) **Transfection**. Add the complexes in a dropwise manner onto the cells growing in complete culture medium and homogenize by gently rocking the plate back and forth to ensure a uniform distribution of the mixture.

4) **Assay.** Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of the transgene expression. We recommend performing assay from 24 to 72h.

Reverse transfection. Prepare the complexes as described above, then transfer them into an empty culture dish or well and then add the cells at twice the recommended cell density.

Other protocols for co-transfection (siRNA/DNA or mRNA/DNA) are also available on our website at www.ozbiosciences.com or by contacting our technical support department (tech@ozbiosciences.com).

OZ Biosciences offers plasmids coding for CAT (#PL00010), **GFP** (#PL00020), **LacZ** (#PL00030), **LUC** (#PL00040) **and SEAP** (#PL00050) **as transfection controls**. These control plasmids are recommended to set up optimization procedure.

3.4. Optimization protocol for DNA transfection

To achieve the highest efficiency, optimize the transfection conditions as follows:

- Vary the HeLaFect (µL) / DNA (µg) ratio from 1/1 to 3/1.
  We recommend trying 1, 1.5, 2 and 3 µL HeLaFect per µg DNA.
- Once the optimal HeLaFect /DNA ratio is found, adjust the DNA quantity according to Table 3.
- Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation times can also be optimized.

Table 3: Suggested range of DNA amounts for optimization (per well).

Tissue Culture Dish format	DNA Quantity (µg)
96 well	0.1 to 0.4
24 well	0.2 to 0.8
6 well	2 to 4

Refer to detailed optimization protocols for 96-, 24- and 6-well the end of this document.

4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

4.1 Quality Controls

To assure the performance of each lot of HeLaFect reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

Specification	Standard Quality Controls
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 15 days.
Biological Activity	Transfection efficiency on HeLa cells. Every lot shall have an acceptance specification of > 85% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Optimization of HeLaFect / DNA ratio.** See section 3.4. 2- **DNA amount.** Use different quantities of DNA with the optimized ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 60 to 80% but most favorable cell density may vary according to the cell subtype; preferably mid-log growth phase. 4- **DNA quality.** Nucleic acid should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins. 5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Use pVectOZ plasmids as controls for transfection. 6- **Cell condition.** 1) Cells in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing DNA / transfection reagent complexes**. It is critical to use serum-free medium or buffer (HBS, PBS) during the complexes preparation. 8- **Culture medium composition.** 1) In some cases, transfection efficiency can be increased in absence of serum. Transfect these cells in serum-free medium during the first 4h. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 18h depending on the readout and the cell. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared each time to avoid aggregation. 11- **Transfection reagent temperature.** Reagents should be at ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials. 2- **Protein expression is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a control plasmid. 3- **DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 5- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(for retrovirus and lentivirus)* / AdenoMag *(for adenovirus and AAV)*
***In vivo* Targeted Delivery**
In vivo PolyMag (*for all nucleic acids*)
In vivo ViroMag *(for all viral vectors)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
FlyFectin *(for Insect cells)*
I-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*

Description
Capture and Concentration of Virus
Mag4C *(to capture, concentrate and conserve viruses)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* & 3DfectIN *(for 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, Luc, CAT, GFP, SEAP
ASSAY KITS
Bradford – Protein Assay Kit
MTT cell proliferation kit
β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+
X-Gal powder 1g / G-418, Sulfate

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com.

6. Purchaser Notification

Limited License

The purchase of the HeLaFect 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). Reagents are intended **for in-house research only** by the buyer. Such use is limited to 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 HeLaFect reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all HeLaFect reagent material and documentation to OZ Biosciences, or by destroying all HeLaFect reagent components. Purchasers are advised to contact OZ Biosciences with the notification that a HeLaFect reagent 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 HeLaFect reagent 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

The HeLaFect 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.

For more information, or for any comments on the terms and conditions of this License, please contact:

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96-well plate - HeLaFect OPTIMIZATION

This protocol is given for HeLaFect (HF) transfection reagent optimization in a 96-well plate culture format. Cells are seeded 24H before transfection in 150 µL of complete medium under standard culture conditions. 4 DNA quantities (0.1 to 0.4 µg) and 4 HF ratios (1:1, 1.5:1, 2:1 and 3:1) are tested.

NOTE (1): Allow reagents to reach room temperature before preparing the complexes (HF/DNA/DMEM).
NOTE (2): Prevent DNA and HF solutions to come into contact with any plastic surface
NOTE (3): DMEM w/o supplement is used for complexes preparation. DNA and HF are diluted in 25 µL each resulting in 50 µL of final transfection volume. Prefer DMEM or PBS over any other medium.

BEFORE BEGINNING, prepare HF dilutions in culture grade H_2O.

- Add **2 µL** HF to **18.0 µL** culture grade H_2O; note the tube (**A**)
- Add **5 µL** HF to **20.0 µL** culture grade H_2O; note the tube (**B**)

1) DNA preparation into 1.5 mL tube. We recommend testing four DNA quantities, preparation for 5 wells:

- 0.1 µg/well: dilute **0.5 µg** DNA in **125 µL** of DMEM alone (or PBS)
- 0.2 µg/well: dilute **1.0 µg** DNA in **125 µL** of DMEM alone (or PBS)
- 0.3 µg/well: dilute **1.5 µg** DNA in **125 µL** of DMEM alone (or PBS)
- 0.4 µg/well: dilute **2.0 µg** DNA in **125 µL** of DMEM alone (or PBS)
- Incubate 5 min at RT

2) HF preparation into a 96-well plate.

a. In (4x4) wells of a 96-well plate add DMEM without supplement according to the following matrix:

HF ratio \ DNA quantities	0.1 µg	0.2 µg	0.3 µg	0.4 µg
1:1	24 µL	23 µL	22 µL	23 µL
1.5:1	23.5 µL	22 µL	22.7 µL	22 µL
2:1	23 µL	23 µL	22 µL	21 µL
3:1	22 µL	22 µL	21.5 µL	23.8 µL

b. In each well, add HF dilutions according to the following matrix:

HF ratio \ DNA quantities	0.1 µg	0.2 µg	0.3 µg	0.4 µg
1:1	1 µL A	2 µL A	3 µL A	2 µL B
1.5:1	1.5 µL A	3 µL A	2.3 µL B	3 µL B
2:1	2 µL A	2 µL B	3 µL B	4 µL B
3:1	3 µL A	3 µL B	4.5 µL B	1.2 µL HF

3) Complexes preparation (in 96w).

- Add **25 µL** of each DNA solution to the corresponding HF dilutions wells
  (ex: into the 4 wells corresponding to 0.1 µg, add 25 µL of the 0.1 µg solution).
- Incubate **20 min** at RT.
- Add **50 µL** of each complex to the cell culture plate according to plate layout.

4) Evaluation of transgene expression.

- Incubate cells at 37°C/5% CO_2
- Monitor transfection efficiency 24 to 48 H after transfection.

NOTE : transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure: www.ozbiosciences.com/pvectoz-7.html

24-well plate – HeLaFect OPTIMIZATION

This protocol is given for HeLaFect transfection (HF) reagent optimization in a 24-well plate culture format. Cells are seeded 24H before transfection in 400 µL of complete medium under standard culture conditions. 4 DNA quantities (0.2 to 0.8 µg) and 4 HF ratios (1:1, 1.5:1, 2:1 and 3:1) are tested.

NOTE (1): Allow reagents to reach room temperature before preparing the complexes (HF/DNA/DMEM).
NOTE (2): Prevent DNA and HF solutions to come into contact with any plastic surface
NOTE (3): DMEM w/o supplement is used for complexes preparation. DNA and HF are diluted in 50 µL each resulting in 100 µL of final transfection volume. Prefer DMEM or PBS than any other medium.

BEFORE BEGINNING, prepare HF dilutions in culture grade H2O.

- Add **6 µL** HF to **24.0 µL** culture grade H2O; note the tube (**A**)

1) DNA preparation into 1.5 mL tube. We recommend testing four DNA quantities, preparation for 5 wells:

- 0.20 µg/well: dilute **1.00 µg** DNA in **250 µL** of DMEM alone (or PBS)
- 0.35 µg/well: dilute **1.75 µg** DNA in **250 µL** of DMEM alone (or PBS)
- 0.50 µg/well: dilute **2.50 µg** DNA in **250 µL** of DMEM alone (or PBS)
- 0.80 µg/well: dilute **4.00 µg** DNA in **250 µL** of DMEM alone (or PBS)
- Incubate 5 min at RT

2) HF preparation into a 96-well plate.

a. In (4x4) wells of a 96-well plate add DMEM without complement according to the following matrix:

DNA quantities

HF ratio	0.2 µg	0.35 µg	0.5 µg	0.8 µg
1:1	49 µL	48.2 µL	47.5 µL	46 µL
1.5:1	48.5 µL	47.3 µL	46.2 µL	48.8 µL
2:1	48 µL	46.5 µL	49 µL	48.4 µL
3:1	47 µL	49 µL	48.5 µL	47.6 µL

b. In each well, add HF dilutions according to the following matrix:

DNA quantities

HF ratio	0.2 µg	0.35 µg	0.5 µg	0.8 µg
1:1	1 µL A	1.8 µL A	2.5 µL A	4 µL A
1.5:1	1.5 µL A	2.7 µL A	3.8 µL A	1.2 µL HF
2:1	2 µL A	3.5 µL A	1 µL HF	1.6 µL HF
3:1	3 µL A	1 µL HF	1.5 µL HF	2.4 µL HF

3) Complexes preparation (in 96-well plate) and transfection (in 24-well plate).

- Add **50 µL** of each DNA solution to the corresponding HF dilutions wells
  (ex: into the 4 wells corresponding to 0.2 µg, add 50 µL of the 0,2 µg solution).
- Incubate **20 min** at RT.
- Add **100 µL** of each complex to the cell culture plate (24-well plate) according to plate layout.

4) Evaluation of transgene expression.

- Incubate cells at 37°C/5% CO_2
- Monitor transfection efficiency 24 to 48 H after transfection.

NOTE : transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure: www.ozbiosciences.com/pvectoz-7.html.

6-well plate - HeLaFect™ OPTIMIZATION

This protocol is given for HeLaFect (HF) transfection reagent optimization in **two** 6-well plates culture format. Cells are seeded 24H before transfection in 2 mL of complete medium under standard culture conditions.
3 DNA quantities (1 to 3 µg) and 4 HF ratios (0.3:1, 0.6:1, 1:1 and 1.3:1) are tested.

NOTE (1): Allow reagents to reach room temperature before preparing the complexes (HF/DNA/DMEM).
NOTE (2): Prevent DNA and HF solutions to come into contact with any plastic surface
NOTE (3): DMEM w/o supplement is used for complexes preparation. DNA and HF are diluted in 250 µL each resulting in 2.5 mL of final transfection volume. Prefer DMEM or PBS than any other medium.

1) DNA preparation into 1.5 mL tube. We recommend testing three DNA quantities, preparation for 5 wells:

- 2.0 µg/well: dilute **10.0 µg** DNA in **1250 µL** of DMEM alone (or PBS)
- 3.0 µg/well: dilute **15.0 µg** DNA in **1250 µL** of DMEM alone (or PBS)
- 4.0 µg/well: dilute **20.0 µg** DNA in **1250 µL** of DMEM alone (or PBS)
- Incubate 5 min at RT

2) HF preparation into a 24-well plate.

a. In (3x4) wells of a 24-well plate add DMEM without complement according to the following matrix:

DNA quantities

HF ratio	2 µg	3 µg	4 µg
1:1	248 µL	247 µL	246 µL
1.5:1	247 µL	245.5 µL	244 µL
2:1	246 µL	244 µL	242 µL
3:1	244 µL	241 µL	238 µL

b. In each well, add HF dilutions according to the following matrix:

DNA quantities

HF ratio	2 µg	3 µg	4 µg
1:1	2 µL HF	3 µL HF	4 µL HF
1.5:1	3 µL HF	4.5 µL HF	6 µL HF
2:1	4 µL HF	6 µL HF	8 µL HF
3:1	6 µL HF	9 µL HF	12 µL HF

3) Complexes preparation (in 24-well plate) and transfection (in 6-well plate).

- Add **250 µL** of each DNA solution to the corresponding HF dilutions wells
  (ex: into the 4 wells corresponding to 2 µg, add 250 µL of the 2 µg solution).
- Incubate **20 min** at RT.
- Add **500 µL** of each complex to the **two** cell culture plates (6-well plates) according to plate layout.

4) Evaluation of transgene expression.

- Incubate cells at 37°C/5% CO_2
- Monitor transfection efficiency 24 to 48 H after transfection.

NOTE : transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure: www.ozbiosciences.com/pvectoz-7.html.

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