Transfection reagent

HeLaFect™

Tee Technology (Triggered Endosomal Escape)

For HeLa cells lineage

Protocol

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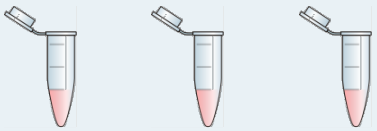
HeLaFect™ Quick Protocol

To find the ideal conditions, HeLaFect™ must be tested at ratios of **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of HeLaFect / 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**

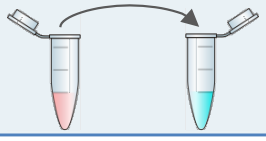


96 well plate	24 well plate	6 well plate
0.25µg in 25µL of serum-free medium or buffer* X 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

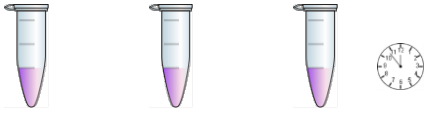
3 **Prepare 3 tubes of HeLaFect (with 3 different amounts of reagent)**

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*

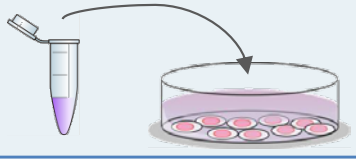
4 **Mix each tube of DNA (step 2) to each tube of HeLaFect (step 3)**



5 **Incubate 15 to 20 min at room temperature**

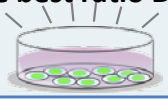


6 **Distribute each mix dropwise onto the cells to insure uniform distribution**



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

8 **Choose the best ratio DNA:HeLaFect**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For HeLa cell lines, 24h before transfection seed the cells 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.
- ✓ Medium or buffer without serum & supplement must be used for the DNA/HeLaFect 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.
- ✓ For doses of HeLaFect less than 1µL, dilute the reagent with deionized water.
- ✓ For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation with fresh medium.

Package content	HF20500: 500µL of HeLaFect HF21000: 1mL of HeLaFect HF25000: 5 x 1mL of HeLaFect
Shipping conditions	Room Temperature
Storage conditions	Store the HeLaFect transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	HeLaFect is a transfection reagent specifically developed for HeLa cell lineage transfection with high efficiency
Important notice	For research use only. Not for use in diagnostic procedures

1. Cell preparation

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 (refer to 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.

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.

Table 1: Suggested cell number (per well)

2. DNA/HeLaFect complexes preparation

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

Tissue Culture Dish	DNA Quantity (µg)	HeLaFect 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	2	2 x 100	2 mL

Table 2: Suggested DNA amount, HeLaFect volume and transfection conditions

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 the DNA solution to the HeLaFect 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

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

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

Protocol | 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 HeLaFect.

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 | Reverse transfection

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

Optimization Protocol

1. General considerations

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.

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

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

2. Optimization protocol | 96-well plate

This protocol is given for HeLaFect 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 HeLaFect ratios (1:1, 1.5:1, 2:1 and 3:1) are tested.

IMPORTANT NOTES

- Allow reagents to reach room temperature before preparing the complexes (HeLaFect/DNA/DMEM).
- Prevent DNA and HeLaFect solutions to come into contact with any plastic surface.
- DMEM w/o supplement is used for complexes preparation. DNA and HeLaFect 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 HELAFECT dilutions in culture grade H_2O.**
  - Add 2 µL HeLaFect to 18.0 µL culture grade H_2O; note the tube (A)
  - Add 5 µL HeLaFect to 20.0 µL culture grade H_2O; note the tube (B)

a. 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

b. HelaFect preparation into a 96-well plate

In (4x4) wells of a 96-well plate add DMEM without supplement according to the Figure 1:

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

Figure 1: volume of DMEM added per well

In each well, add HeLaFect dilutions according to the Figure 2:

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

Figure 2: volume of HelaFect added per well

c. Complexes preparation (in 96w)

- Add 25 µL of each DNA solution to the corresponding HeLaFect 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.

d. Evaluation of transgene expression

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

NOTE: transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure!

3. Optimization protocol | 24-well plate

This protocol is given for HeLaFect transfection 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 HeLaFect ratios (1:1, 1.5:1, 2:1 and 3:1) are tested.

IMPORTANT NOTES

- Allow reagents to reach room temperature before preparing the complexes (HeLaFect/DNA/DMEM).
- Prevent DNA and HeLaFect solutions to come into contact with any plastic surface.
- DMEM w/o supplement is used for complexes preparation. DNA and HeLaFect 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 HeLaFect dilutions in culture grade H2O.**
  - Add 6 µL HeLaFect to 24 µL culture grade H2O; note the tube (A)

a. DNA preparation into 1.5 mL tube

We recommend testing four DNA quantities, preparation for 5 wells:

- 0.20 µg/well: dilute 1 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 2.5 µg DNA in 250 µL of DMEM alone (or PBS)
- 0.80 µg/well: dilute 4 µg DNA in 250 µL of DMEM alone (or PBS)
- Incubate 5 min at RT

b. HELAFECT preparation into a 96-well plate

In (4x4) wells of a 96-well plate add DMEM without supplement according to the Figure 3:

HF ratio \ DNA quantities	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

F

In each well, add HeLaFect dilutions according to the following matrix:

HF ratio \ DNA quantities	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

Figure 4: volume of HeLaFect (HF) added per well

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

- Add 50 µL of each DNA solution to the corresponding HeLaFect 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.

d. Evaluation of transgene expression

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

NOTE: transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure!

4. Optimization protocol | 6-well plate

This protocol is given for HeLaFect 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 HeLaFect ratios (0.3:1, 0.6:1, 1:1 and 1.3:1) are tested.

IMPORTANT NOTES

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

a. DNA preparation into 1.5 mL tube

We recommend testing three DNA quantities, preparation for 5 wells:

- 2 µg/well: dilute 10 µg DNA in 1250 µL of DMEM alone (or PBS)
- 3 µg/well: dilute 15 µg DNA in 1250 µL of DMEM alone (or PBS)
- 4 µg/well: dilute 20 µg DNA in 1250 µL of DMEM alone (or PBS)
- Incubate 5 min at RT

b. HeLaFect preparation into a 24-well plate

In (3x4) wells of a 24-well plate add DMEM without complement according to the Figure 5:

HF ratio \ DNA quantities	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

Figure 5: volume of DMEM added per well

In each well, add HeLaFect dilutions according to the Figure 6:

HF ratio \ DNA quantities	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

Figure 6: volume of HeLaFect (HF) added per well

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

- Add 250 µL of each DNA solution to the corresponding HeLaFect 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.

d. Evaluation of transgene expression

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

NOTE: transfection efficiency highly depends on plasmid quality, use one of our pVectOZ- control plasmids for a better optimization procedure!