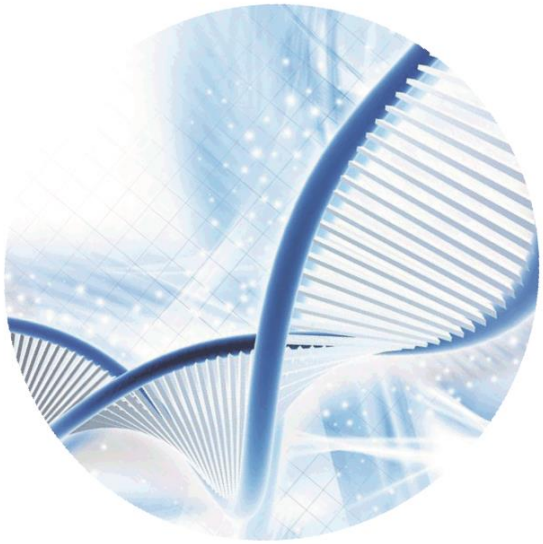

Transfection reagent

COSFect™

Tee Technology (Triggered Endosomal Escape)

COS cell lines

Protocol

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The art of delivery systems

COSFect Quick Protocol

To find the ideal conditions, COSFect must be tested at ratios **1 µL/µg**, **2 µL/µg** and **3 µL/µg** (µL of COSFect / µg of DNA). For the DNA quantity, we suggest **0.25 µg** per well in 96-well, **0.4 µg** per well in 24-well and **1 µg** per well in 6-well.

1

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

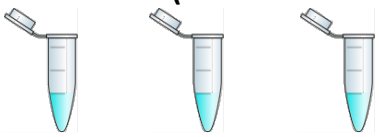
2

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	0.4µg in 50µL of serum-free medium or buffer* x 3	1µg in 100µL of serum-free medium or buffer* x 3

3

Prepare 3 tubes of COSFect (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*	0.4µL/0.8µL/1.2µL in 50µL of serum-free medium or buffer*	1µL/2µL/3µL in 100µL of serum-free medium or buffer*

4

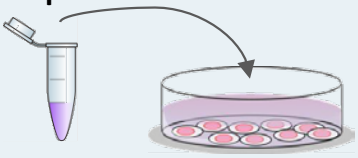
Mix each tube of DNA (step 2) to each tube of COSFect (step 3)

5

Incubate 20 min at room temperature

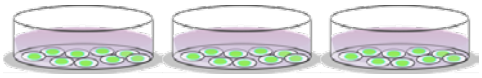
6

Distribute each mix dropwise onto the cells to insure uniform distribution



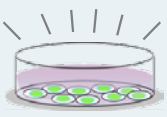
7

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



8

Choose the best ratio DNA:COSFect



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 COS cells, 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/COSFect 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 COSFect less than 1µL, dilute the reagent with deionized water.

- ✓ For some cells, 24 hours 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 3-4 hours or 24 hours incubation with fresh medium.

Package content	CF10500: 500µL of COSFect CF11000: 1mL of COSFect CF12500: 5 x 1mL of COSFect
Shipping conditions	Room Temperature
Storage conditions	Store the COSFect transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	COSFect is a high efficiency transfection reagent specifically developed for COS cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cell Preparation

One day before transfection prepare the cells according to the Table 1 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 (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	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/COSFect complexes preparation

a. *COSFect*: Vortex the reagent and dilute the indicated quantity of COSFect (refer to able 1) in 25 to 100 µL of culture medium without serum and supplement.

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 COSFect 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 COSFect / 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.

Tissue Culture Dish	DNA Quantity (µg)	COSFect Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.2	0.4	2 x 25	200 µL
24 well	0.4	0.8	2 x 50	500 µL
6 well	1	2	2 x 100	2 mL

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

Protocol | DNA 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 COSFect.

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.

Protocol | Optimization

1. General considerations

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

- Vary the COSFect (µL) / DNA (µg) ratio from 0.6/1 to 2.6/1. We recommend trying 0.6, 1.2, 2.0 and 2.6 µL COSFect per µg DNA.
- Once the optimal CosFect/DNA ratio 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	DNA Quantity (µg)
96 well	0.125 to 0.25
24 well	0.2 to 0.8
6 well	1 to 3

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

2. Optimization protocols

a. 96 well plate - COSFect OPTIMIZATION

This protocol is given for COSFect 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 (0.125 to 0.25 µg) and 4 COSFect ratios (0.6:1, 1.2:1, 2:1 and 2.6:1) are tested.

IMPORTANT NOTES

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

BEFORE BEGINNING, prepare COSFect dilutions in culture grade H2O

- Add 2 µL COSFect to 24.6 µL culture grade H2O; note the tube (A)
- Add 2 µL COSFect to 20.2 µL culture grade H2O; note the tube (B)
- Add 2 µL COSFect to 14.6 µL culture grade H2O; note the tube (C)
- Add 2 µL COSFect to 12.3 µL culture grade H2O; note the tube (D)

i) DNA preparation into 1.5 mL tube

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

- 0.125 µg/well: dilute 0.625 µg DNA in 125 µL of DMEM alone (or PBS)
- 0.150 µg/well: dilute 0.750 µg DNA in 125 µL of DMEM alone (or PBS)
- 0.200 µg/well: dilute 1.000 µg DNA in 125 µL of DMEM alone (or PBS)
- 0.250 µg/well: dilute 1.250 µg DNA in 125 µL of DMEM alone (or PBS)
- Incubate 5 min at RT

ii) COSFect preparation into a 96-well plate

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

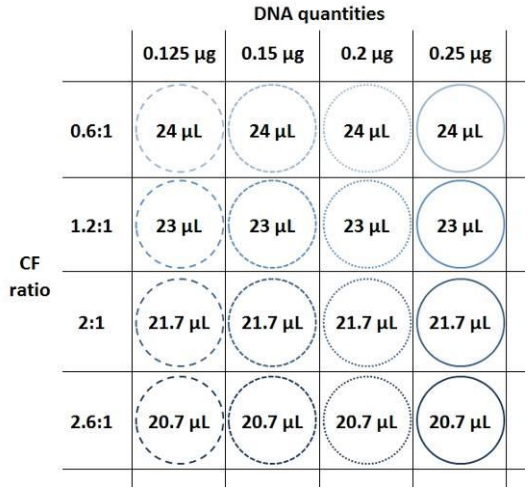


Figure 1: volume of DMEM added per well (CosFect, CF)

b. In each well, add COSFect dilutions according to the Figure 2

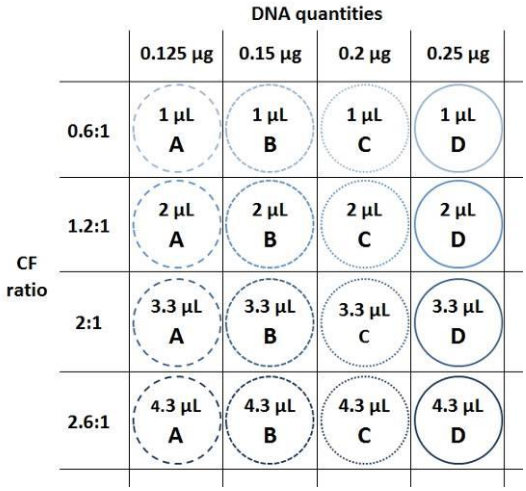


Figure2: volume of Cosfect (CF) added per well

iii) Complexes preparation (in 96w)

- Add **25 µL** of each DNA solution to the corresponding COSFect dilutions wells (ex: into the 4 wells corresponding to 0.125 µg, add 25 µL of the 0,125µg solution)
- Incubate **20 min** at RT
- Add **50 µL** of each complex to the cell culture plate according to plate layout

iv) 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 our pVectOZ- plasmid for a better optimization procedure.

b. 24-well plate - COSFect OPTIMIZATION

This protocol is given for COSFect 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 COSFect ratios (0.6:1, 1.2:1, 2:1 and 2.6:1) are tested.

IMPORTANT NOTES

- Allow reagents to reach room temperature before preparing the complexes (COSFect /DNA/DMEM).
- Prevent DNA and COSFect solutions to come into contact with any plastic surface
- DMEM w/o supplement is used for complexes preparation. DNA and COSFect 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 COSFect dilutions in culture grade H2O

- Add 4 µL COSFect to 29.2 µL culture grade H2O; note the tube (A)
- Add 5 µL COSFect to 8.5 µL culture grade H2O; note the tube (B)
- Add 7 µL COSFect to 7.5 µL culture grade H2O; note the tube (C)

i) DNA preparation into 1.5 mL tube

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

- 0.2 µg/well: dilute 1 µg DNA in 250 µL of DMEM alone (or PBS)
- 0.4 µg/well: dilute 2 µg DNA in 250 µL of DMEM alone (or PBS)
- 0.6 µg/well: dilute 3 µg DNA in 250 µL of DMEM alone (or PBS)
- 0.8 µg/well: dilute 4 µg DNA in 250 µL of DMEM alone (or PBS)
- Incubate 5 min at RT

ii) COSFect preparation into a 96-well plate

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

CF ratio \ DNA quantities	0.2 µg	0.4 µg	0.6 µg	0.8 µg
0.6:1	49 µL	48 µL	49 µL	49 µL
1.2:1	48 µL	46 µL	48 µL	48 µL
2:1	46.7 µL	43.4 µL	46.7 µL	46.7 µL
2.6:1	45.7 µL	41.4 µL	45.7 µL	45.7 µL

Figure 3: volume of DMEM added per well (CosFect, CF)

b. In each well, add COSFect dilutions according to the Figure 4

CF ratio \ DNA quantities	0.2 µg	0.4 µg	0.6 µg	0.8 µg
0.6:1	1 µL A	2 µL A	1 µL B	1µL C
1.2:1	2 µL A	4 µL A	2 µL B	2 µL C
2:1	3.3 µL A	6.6 µL A	3.3 µL B	3.3 µL C
2.6:1	4.3 µL A	8.6 µL A	4.3 µL B	4.3 µL C

Figure 4: volume of CosFect (CF) added per well

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

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

iv) 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 our pVectOZ- plasmid for a better optimization procedure.

c. 6-well plate - COSFect OPTIMIZATION

This protocol is given for COSFect 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 COSFect ratios (0.6:1, 1.2:1, 2:1 and 2.6:1) are tested.

IMPORTANT NOTES

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

BEFORE BEGINNING, prepare COSFect dilutions in culture grade H2O

- Add 6.0 µL COSFECT to 6.0 µL culture grade H2O; note the tube (A)

i) DNA preparation into 1.5 mL tube

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

- 1.0 µg/well: dilute 05.0 µg DNA in 1250 µL of DMEM alone (or PBS)
- 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)
- Incubate 5 min at RT

ii) COSFect preparation into a 24-well plate

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

CF ratio \ DNA quantities	1 µg	2 µg	3 µg
0.6:1	248.8 µL	247.6 µL	246.4 µL
1.2:1	247.6 µL	247.6 µL	246.4 µL
2:1	248 µL	246 µL	244 µL
2.6:1	248.4 µL	244.8 µL	242.8 µL

Figure 5: volume of DMEM added per well (CosFect, CF)

b. In each well, add COSFect dilutions according to the Figure 6

CF ratio \ DNA quantities	1 µg	2 µg	3 µg
0.6:1	1.2 µL A	2.4 µL A	3.6 µL A
1.2:1	2.4 µL A	2.4 µL CF	3.6 µL CF
2:1	2 µL CF	4 µL CF	6 µL CF
2.6:1	2.6 µL CF	5.2 µL CF	7.2 µL CF

Figure 6: volume of CosFect (CF) added per well

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

- Add 250 µL of each DNA solution to the corresponding COSFect dilutions wells (ex: into the 4 wells corresponding to 1 µg, add 250 µL of the 1 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.

iv) 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 our pVectOZ- plasmid for a better optimization procedure.