Transfection reagent

EcoTransFect™

Lipofection Reagent
Cell lines

Protocol

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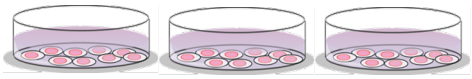
The art of delivery systems

EcoTransfect™ Quick Protocol

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

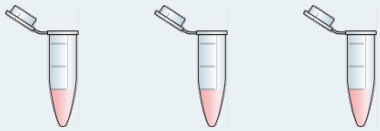
1

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

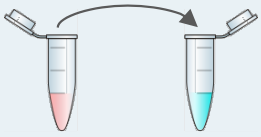
3

Prepare 3 tubes of EcoTransfect (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*	1µL/2µL/3µL in 50µL of serum-free medium or buffer*	3µL/6µL/9µL in 100µL of serum-free medium or buffer*

4

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

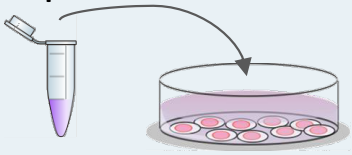


5

Incubate 20 min at RT

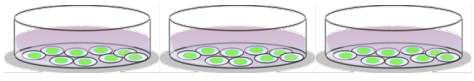
6

Distribute each mix dropwise onto the cells to insure uniform distribution



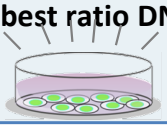
7

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



8

Choose the best ratio DNA:EcoTransfect



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 cell lines, 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.
- ✓ **Medium or buffer without serum & supplement** must be used for the DNA/EcoTransfect 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 medium with fresh pre-warm medium 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.
- ✓ Prevent EcoTransfect 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 EcoTransfect directly into the solution.

Package content	ET10500: 500µL of EcoTransfect ET11000: 1mL of EcoTransfect ET13000: 3 x 1mL of EcoTransfect
Shipping conditions	Room Temperature
Storage conditions	Store the EcoTransfect transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	EcoTransfect Transfection Reagent is an economical Lipofection reagent dedicated to the transfection of popular cell lines.
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA or shRNA vectors transfection

1. Cell Preparation

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) 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 transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Tissue Culture Dish	Adherent Cell Number	DNA Quantity (µg)	Ecotransfect Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.25	0.5	2 x 25	200 µL
24 well	0.5 – 1 x 10^5	1	2	2 x 50	500 µL
6 well	2 – 5 x 10^5	3	6	2 x 100	2 mL

Table 1: Suggested DNA amount, Ecotransfect volume and transfection conditions

2. DNA/Ecotransfect complexes preparation

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

b. *DNA*: Dilute the indicated quantity of DNA (see Table 1) in 25 to 100 µL of culture medium without serum and supplement.

c. Add DNA solution to Ecotransfect 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 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.

NOTE: in case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium.

IMPORTANT OBSERVATION FOR PROTEIN PRODUCTION OVER 24H

In case of protein production experiment over 24h, we recommend using two times more amounts of DNA per well to yield maximal levels of protein.

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 Ecotransfect.

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 | stable transfection

The same protocol can be used to produce stably transduced cells except that 48h 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.

Optimization Protocol

We advise you to optimize your transfection conditions in order to get the best out of EcoTransfect™. Several parameters can be optimized:

- Ratio of EcoTransfect™ to nucleic acid
- Quantity of nucleic acid
- Cell density
- Culture medium composition (+/- serum)

1. EcoTransfect™ / DNA ratio

This is a main optimization parameter. EcoTransfect™ has to be used in excess compared to DNA but the optimal ratio will depend on the cell line and the vessel used. It is particularly true for 96 well plates because of adsorption processes. For optimization, first maintain a fixed quantity of DNA (according to the size of your culture dish or cell number) and then vary the ratio of EcoTransfect™ reagent to DNA over the suggested range in the Table 2. You can test ratios from 1 to 5 µl of EcoTransfect™ reagent per 1 µg DNA.

Tissue Culture Dish	DNA Quantity (µg)	EcoTransfect™ Volume (µL)	EcoTransfect™ Volume (µL) proposed interval
96 well	0.2	0.2 - 1	0.2 – 0.4 – 0.6 – 0.8 – 1
24 well	1	1 – 5	1 – 2 – 3 – 4 - 5
6 well	3	3 - 15	3 – 6 – 9 – 12 – 15

Table 2: Suggested range of EcoTransfect™ for optimization

2. Quantity of DNA

To achieve the optimum transfection efficiency, the amount of nucleic acid used (DNA) can be optimized. Keep the number of cells and the incubation time constant and adjust the quantity of nucleic acid while maintaining a fixed ratio of EcoTransfect reagent to DNA (refer to Table 3)

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.1 – 0.8	200 µl
24 well	0.5 – 2	500 µl
6 well	2 – 8	2 mL

Table 3: Suggested range of DNA amounts for optimization

Following these two steps process, culture medium compositions, cell number, incubation times can also be optimized.

3. Cell number

The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used. Thus, the next step is to use the optimize ratio and DNA amount obtained previously and varied the cell number to be assayed.

For stable transfection, cells can be seeded with lower density. 48 to 72 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection.

4. Effect of serum /Transfection volume

Almost all cell lines transfected with EcoTransfect™ showed excellent results if serum is present during the transfection. Some cell lines may behave differently and transfection efficiency can be increased without serum or under reduced serum condition.

IMPORTANT CONSIDERATIONS

Remember that presence of serum during complex formation is strictly prohibited, as the serum will inhibit their formation. Transfection efficiency is attained when the initial 3-4 hours of incubation is done. Consequently, the cells may be kept in serum-free medium during the first 4 hours of transfection, then replace it by a culture medium containing serum or just add serum to the wells according to your standard culture condition after this period.

5. Incubation time

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

Additional products for your transfection experiments

- **COSFect** - a transfection reagent dedicated to COS cells lineage
- **HeLaFect** - a transfection reagent dedicated to HeLa cells lineage

Purchaser Notification

Limited License

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