

Tee Technology (Triggered Endosomal Escape) DNA Delivery

To find the ideal conditions, EcoTransfect™ must be tested at ratios of **1 $\mu\text{L}/\mu\text{g}$** , **2 $\mu\text{L}/\mu\text{g}$** and **3 $\mu\text{L}/\mu\text{g}$** (μL of EcoTransfect / μg of DNA). For the DNA quantity, we suggest **0.25 μg** per well in 96-well, **1.0 μg** per well in 24-well and **3 μg** per well in 6-well.

1. Prepare 3 wells: seed the cells to be at 70% confluent on the day of transfection



96-well plate
0.05-0.2 x 1.10^5 cells



24-well plate
0.5-1 x 1.10^5 cells



6-well plate
2-5 x 1.10^5 cells

2. Prepare 3 identical tubes of DNA



96-well plate

0.25 μg in 25 μL of serum-free medium or buffer **X 3**

24-well plate

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

6-well plate

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

3. Prepare 3 tubes of EcoTransfect



96-well plate

0.25 μL /0.5 μL /0.75 μL in 25 μL of serum-free medium or buffer

24-well plate

1 μL /2 μL /3 μL in 50 μL of serum-free medium or buffer

6-well plate

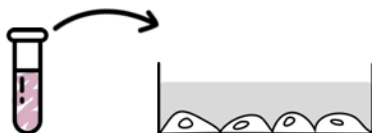
3 μL /6 μL /9 μL in 100 μL of serum-free medium or buffer

4. Mix each DNA suspension to each tube of EcoTransfect



Incubate 15—20 min at RT

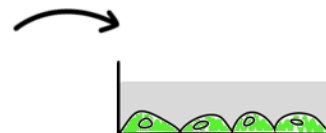
5. Distribute each mix dropwise onto the cells to insure uniform distribution



Distribute each preparation of complexes in each well



Incubate 24 to 72 h at 37°C



Chose the best ratio DNA:EcoTransfect

Contact us regarding protocols for DNA co-transfection, optimization procedure, protein production, stable transfections at: tech@ozbiosciences.com or visit our website: www.ozbiosciences.com.

Before you begin

EcoTransfect™ is an economic and powerful reagent dedicated to the transfection of the most commonly used cell lines.

Protocol | General considerations

- Allow reagents to reach room temperature.
- Use medium w/o any supplement for preparation of complexes, avoid using RPMI.
- Sensitive cells may need a medium change 3-4 h after transfection. Optionally, replace medium 24h post-transfection.
- Prevent EcoTransfect reagent solution to encounter any plastic surface that could result in material lost by adsorption: first, add serum-free culture medium to the tube and then, EcoTransfect.

1. Cells preparation:

One day before transfection prepare the cells according to table 1 (recommended 70% confluence).

| Cell culture format | Adherent cell nb. / well | DNA quantity | EcoTransfect volume | Dilution volume | Transfection volume |
|---------------------|------------------------------|--------------|---------------------|-----------------|---------------------|
| 96-well | 0.05-0.2 x 1.10 ⁵ | 0.25 µg | 0.5 µL | 2 x 25 µL | 200 µL |
| 24-well | 0.5-1 x 1.10 ⁵ | 1 µg | 2 µL | 2 x 50 µL | 500 µL |
| 6-well | 2-5 x 1.10 ⁵ | 3 µg | 6 µL | 2 x 100 µL | 2 mL |

Table 1: suggested DNA amount, EcoTransfect volume and transfection conditions per well.

2. Preparation of DNA/EcoTransfect complexes:

a. **DNA:** Dilute the indicated quantity of DNA in **25 to 100 µL** of culture medium w/o supplement (refer to Table 1).

b. **EcoTransfect:** vortex the reagent and dilute the indicated quantity of EcoTransfect in **25 to 100 µL** of culture medium w/o supplement (refer to Table 1).

c. Add DNA solution to EcoTransfect solution, mix gently by carefully pipetting up and down.

d. Incubate the mixture at room temperature for **15-20 min.**

⚠ 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 CO₂ incubator under standard conditions until evaluation of transgene expression.

Protocol | Stable transfection | Protein production | co- transfections | Optimization

Contact us regarding protocols for DNA co-transfection, optimization procedure, stable transfections, protein production... or any technical question:

✉ - tech@ozbiosciences.com.

Use, handling and storage

For Research Use Only. Not for use in humans. Not for use in diagnostic or therapeutic purposes.

Shipping conditions: Room Temperature

Storage conditions EcoTransfect: 4°C

Shelf life: 1 year from the date of purchase

Kit contents

ET10500: 500 µL of EcoTransfect

ET11000: 1 mL of EcoTransfect

ET13000: 3x1 mL of EcoTransfect

Certificate of analysis on demand.

Related Products

| Ref | Description |
|----------|---|
| #DG81000 | Dreamfect™ Gold transfection reagent |
| #PG61000 | PolyMag Neo for hard-to-transfect cells |
| #PL00020 | pVectOZ transfection plasmids – positive controls |

Purchaser Notification | Conditions of Sale

This product is sold in accordance with our general conditions of sale that you can find on our website: <https://ozbiosciences.com/content/3-terms-and-conditions>.