

To find the ideal conditions, DreamFect™ Stem must be tested at ratios of **2 $\mu\text{L}/\mu\text{g}$** , **3 $\mu\text{L}/\mu\text{g}$** and **4 $\mu\text{L}/\mu\text{g}$** (μL of DreamFect Stem / μg of DNA). For the DNA quantity, we suggest **0.125 μg** per well in 96-well, **0.5 μg** per well in 24-well and **2 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
6-12 x 1.10^3 cells



24-well plate
4-8 x 1.10^4 cells



6-well plate
2-4 x 1.10^5 cells

2. Prepare 3 identical tubes of DNA



96-well plate

0.125 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

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

3. Prepare 3 tubes of DreamFect Stem



96-well plate

0.25 μL /0.375 μL /0.5 μL in 25 μL of serum-free medium or buffer

24-well plate

1 μL /1.5 μL /2 μL in 50 μL of serum-free medium or buffer

6-well plate

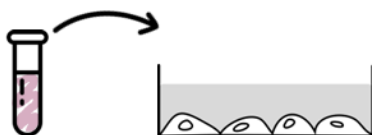
4 μL /6 μL /8 μL in 100 μL of serum-free medium or buffer

4. Mix each DNA suspension to each tube of DreamFect Stem



Incubate 20 min at RT

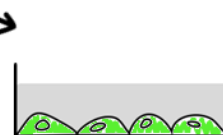
5. Distribute each mix dropwise onto the cells to insure uniform distribution



Distribute each preparation of complexes in each well



Incubate 24 to 72 h at 37°C RT



Chose the best ratio DNA: DreamFect Stem

Contact us regarding protocols for DNA transfection in suspension cells, optimization procedures or for co-transfection at: tech@ozbiosciences.com or visit our website: www.ozbiosciences.com.

Before you begin

DreamFect™ Stem is a lipopolyamine formulation specifically designed to achieve high transfection efficiency in a wide variety of cell lines and primary cells.

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 24 h post-transfection.

1. Cells preparation:

One day before transfection prepare the cells according to table 1. The cells should be 50-70% confluence at the time of transfection (40% for embryonic stem cells).

Cell culture format	Adherent cell nb. / well
96-well	6-12 x 1.10 ³
24-well	4-8 x 1.10 ⁴
6-well	2-4 x 1.10 ⁵

Table 1: Cell number suggested per well.

2. Preparation of DNA/DreamFect Stem complexes:

a. **DNA:** Dilute the indicated quantity of DNA in **25 to 100 µL** of culture medium w/o supplement (refer to Table 2).

b. **DreamFect Stem:** vortex the reagent and dilute the indicated quantity of DreamFect Stem in **25 to 100 µL** of culture medium w/o supplement (refer to Table 2).

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

d. Incubate the mixture at room temperature for **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.

Cell culture format	DNA quantity	DreamFect Stem volume	Dilution volume	Transfection volume
96-well	0.125 µg	0.375 µL	2 x 25 µL	200 µL
24-well	0.5 µg	1.5 µL	2 x 50 µL	500 µL
6-well	2 µg	6 µL	2 x 100 µL	2 mL

Table 2: Recommended DNA amounts, DreamFect Stem volumes and transfection conditions.

Important observations

For ES cells growing on feeder cells or in feeder-free system, double the amount of DNA and reagent (i.e. 1 µg for 3 µL DreamFect Stem in a 24-well plate, 2 µg for 6 µL DreamFect Stem in a 12-well plate or 3 to 4 µg of DreamFect Stem in a 6-well plate).

Protocol | Suspension cells | Co-transfections | Protein production

Contact us regarding protocols for DNA transfection in suspension cells, co-transfections, protein production, optimization procedure... 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 DreamFect Stem: -20°C

Shelf life: 1 year from the date of purchase

We recommend minimizing freeze-thaw cycles to preserve DreamFect Stem integrity.

Kit contents

ST30500: 500 µL of DreamFect Stem

ST31000: 1 mL of DreamFect Stem

Certificate of analysis on demand.

Related Products

Ref	Description
#DG80500	DreamFect Gold transfection reagent
#LS21000	Lullaby Stem transfection reagent for siRNA
#GXS0003	Senescence Assay Kit

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>.