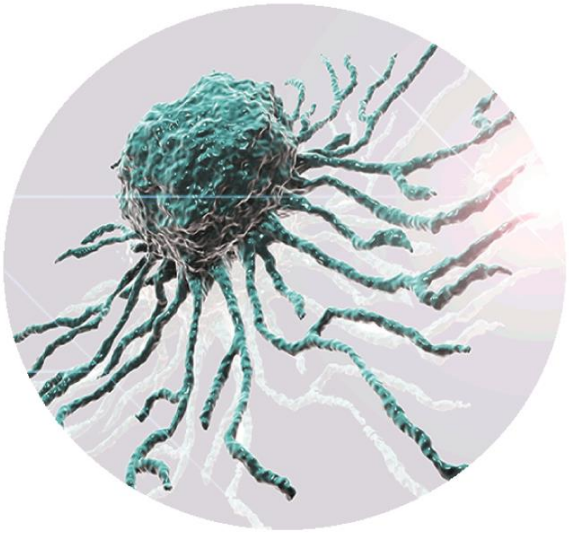

Stem Cell Applications

DreamFect™ Stem

Tee Technology (Triggered Endosomal Escape)

Multipotent stem cells

Protocol

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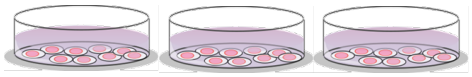
The art of delivery systems

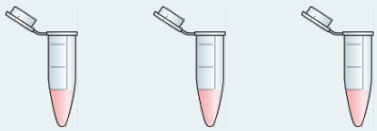
DreamFect™ Stem Quick Protocol

To find the ideal conditions, DreamFect™ Stem must be tested at ratios of **2 µL/µg**, **3 µL/µg** and **4 µL/µg** (µL of DreamFect Stem / µg of DNA). For the DNA quantity, we suggest **0.125 µg** per well in 96-well, **0.5 µg** / well in 24-well and **2 µg** / well in 6-well.

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



2 **Prepare 3 identical tubes of DNA**

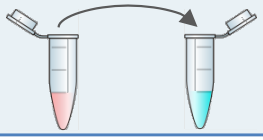


96 well plate	24 well plate	6 well plate
0.125µg in 25µL of serum-free medium or buffer* X 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

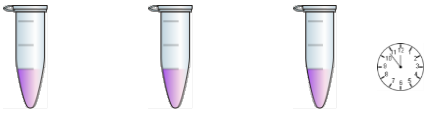
3 **Prepare 3 tubes of DreamFect™ Stem (with 3 different amounts of reagent)**

96 well plate	24 well plate	6 well plate
0.25µL/0.375µL/0.5µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	4µL/6µL/8µL in 100µL of serum-free medium or buffer*

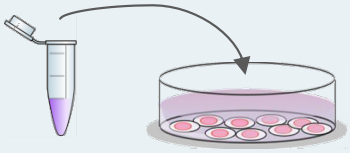
4 **Mix each tube of DNA (step 2) to each tube of DreamFect™ Stem (step 3)**



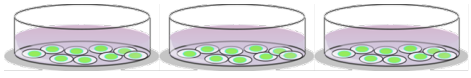
5 **Incubate 15 to 20 min at room temperature**



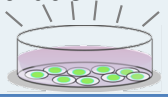
6 **Distribute each mix dropwise onto the cells to insure uniform distribution**



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



8 **Choose the best ratio DNA:DreamFect™ Stem**



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/DreamFect Stem 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, dilute the reagent with deionized water.

- ✓ 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.

DreamFect Stem Reagent | Specifications

Package content	ST30500: 500µL of DreamFect Stem ST31000: 1mL of DreamFect Stem
Shipping conditions	Room Temperature
Storage conditions	Store the DreamFect Stem transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	DreamFect™ Stem Transfection Reagent is a powerful reagent allowing multipotent stem cells transfection with high efficiency and very low toxicity.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

- Seed the cells the day prior transfection. The cells should be at 50-70% confluent at the time of transfection (40% for embryonic stem cells). See the suggested cell number in the Table 1.
- Use cells with a low number of passages.

Culture vessel	Number of adherent cells	DNA Quantity	DreamFect Stem Volume	Dilution Volume	Transfection Volume
96-well	$6 – 12 \times 10^3$	0.125 µg	0.375 µL	2 x 25 µL	200 µL
24-well	$4 – 8 \times 10^4$	0.5 µg	1.5 µL	2 x 50 µL	500 µL
6-well	$2 – 4 \times 10^5$	2 µg	6 µL	2 x 100 µL	2 mL

Table 1: Suggested number of cells to seed DNA amount, DreamFect Stem volume and transfection conditions

2. DNA/DreamFect Stem complexes preparation

a. *DreamFect Stem*: Vortex the reagent and dilute the indicated quantity of DreamFect Stem 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 the DNA solution to the DreamFect Stem solution by vigorous pipetting or brief vortexing and incubate at room temperature for 20 min.

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.

IMPORTANT OBSERVATIONS

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

1. Cells Preparation

The day prior transfection split the cell, in order to have them in an exponential phase of growing.

2. DNA/DreamFect Stem complexes preparation

a. *DNA*: Dilute the indicated quantity of DNA (refer to Table 2) in 25 to 50 µL of culture medium without serum and supplement

Cell culture dish	Suspension cells number	DNA quantity	DreamFect Stem Volume	Dilution volume	Transfection volume
96 well plate	0.5 – 1 x 10^5	0.25 µg	0.75 µL	2 x 25	100 µL
24 well plate	2 – 4 x 10^5	1 µg	3 µL	2 x 50	250 µL
6 well plate	10 – 15 x 10^5	3 µg	9 µL	2 x 50	1 mL

Table2: Transfection conditions suggested for suspension cells

b. *DreamFect Stem*: dilute the indicated quantity of DreamFect Stem (see Table 1) in 25 to 100 µL of culture medium without serum and supplement (refer to Table 2).

c. Add the DNA solution to the DreamFect Stem solution by vigorous pipetting or brief vortexing and incubate at room temperature for 20 min.

NOTE: The diluted solutions should be combined within 5 min.

3. Transfection

a. During complexes incubation, prepare your cells preferentially in serum-free medium. Seed the cells in half of the volume currently use (refer to table 2).

b. Add the mixture dropwise to the cells.

c. Incubate the cells at 37°C in CO2 incubator under standard conditions for 4h.

d. Add culture medium containing 20% serum (same volume as transfection volume).

e. Incubate your cells at 37°C under standard conditions until evaluation of your transgene expression (24 to 72 h following transfection).

IMPORTANT OBSERVATIONS

- Transfections of cells in suspension are optimal when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.

- From our experiences, the key feature is to promote as much as you can the contact between the cells and the transfection complexes. Consequently, here are a few additional propositions:
    - Option 1, concentrate your cells: When the complexes are forming prepare your cells. Spin down the cells, resuspend them at 10×10^6 cells / mL in medium (serum free) and transfer the appropriate cell number to your well according to Table 2. Thereafter, mix the complexes with the cells, incubate 15 min and complete the culture medium as indicated in Table 2.
    - Option 2, promote contact by centrifugation: Centrifuge the plate after having mixed the cells with the transfection complexes for 2-3 min at around 1000-1200 rpm.

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 1.5 µL of DreamFect Stem.

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 | siRNA

1. Cell Preparation siRNA/DreamFect Stem complexes preparation

The day prior transfection, prepare the cells as described in Table 1. Generally, siRNA transfection requires lower cell density than DNA transfection. The correct choice of optimal density depends on the planned time between transfection and gene knockdown analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

2. siRNA/DreamFect Stem complexes preparation

The siRNA and DreamFect Stem solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (see Table 3) of culture medium without serum and antibiotics.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 3: Suggested dilution procedure and amount of siRNA to test

b. *DreamFect Stem preparation*. Put 0.5 to 5 µL of DreamFect Stem in 50 or 100 µL of culture medium without serum and antibiotics (refer to Table 4 for corresponding volumes of DreamFect Stem).

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Final siRNA concentration	*Amount of DreamFect Stem (µl)*		
10 nM	0.5	2	8
20nM	1	3	10
≥ 50 nM	1	4	14

Table 4: Recommended amount of DreamFect Stem per nM of siRNA used

c. Add the siRNA solution onto the DreamFect Stem reagent. Mix gently by carefully pipetting up and down and incubate the mixture for 15 min at room temperature. Do not vortex or centrifuge!

3. Transfection

a. Add the complexes onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis. We recommend 24H for RNA analysis and 48H to 72H for protein knockdown analyses.

NOTES:

- Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection
- For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- If cells are very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation.

Optimization Protocol

We recommend optimization of the transfection protocol for each combination of plasmid and stem cell type used in order to get the best out of DreamFect™ Stem. Several parameters can be optimized:

- Ratio of DreamFect™ Stem to nucleic acid
- Dose of nucleic acid used
- Cell type and cell density
- Culture medium composition (+/- serum) and reagent / nucleic acid complex medium
- Incubation time

We recommend that you optimize one parameter at a time while keeping the other parameters (cell number, incubation time etc.) constant. The two most critical variables are the ratio of DreamFect™ Stem reagent to DNA and the quantity of DNA.

1. DreamFect™ Stem / DNA ratio

This is an important optimization parameter. DreamFect™ Stem has to be used in slight excess compare 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 amount of DreamFect™ Stem reagent over the suggested range in the Table 5. **You can test ratios from 1 to 6 µL of DreamFect™ Stem reagent per 1 µg DNA**.

Tissue Culture Dish	DNA Quantity (µg)	DreamFect Stem Volume (µL)
96 well	0.125	0.1 – 0.6
24 well	0.5	0.5 – 3
6 well	2	2 – 12

ⓘ For ES cells growing on feeders cells or in feeders free system, double the amount of DNA and reagent

Table 5: Suggested range of DreamFect™ Stem for optimization

2. Quantity of DNA

To achieve the optimum transfection efficiency, the amount of nucleic acid used 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 DreamFect™ Stem reagent to DNA (refer to Table 6).

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.1 – 0.5	200 µL
24 well	0.25 – 2	500 µL
6 well	2 – 10	2 mL

ⓘ For ES cells growing on feeders cells or in feeders free system, double the amount of DNA and reagent

Table 6: Suggested range of DNA amounts for optimization

Thereafter, culture medium compositions, cell number, incubation times can also be optimized.

3. DreamFect™ Stem / Nucleic acid complex medium

Several tests demonstrated that the use of PBS to prepare complexes instead of serum- and antibiotic-free medium leads to more reproducible transfections and in some cases higher efficiency, particularly with lower volumes of transfection reagent. PBS composition: 137mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4 and 6.5mM Na_2HPO_4 x 2 H_2O; pH7.4.

4. Cell number

The cell proliferating rate is also a critical parameter in particular for stem cells and the optimal confluency has to be adjusted according to the cells used. Thus, the next step is to use the optimized parameters obtained previously and vary the cell number to be assayed.

NOTE: The addition of the transfection complex directly to freshly seeded cells can result in a considerable increase of transfection efficiency.

5. Effect of serum /Transfection volume

Almost all stem cell types transfected with DreamFect™ Stem showed superior results if serum is present during the transfection. Some stem cells may behave differently and transfection efficiency can be increased without serum or under reduced serum condition. **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 h of incubation is done. Consequently, the cells may be kept in serum-free or reduced serum conditions during the first 4 h of transfection. If you use serum-free medium, replace it by a culture medium containing serum or just add serum to the wells according to your standard culture condition after this period. To increase the efficiency of transfection you can reduce the transfection volume.

6. Incubation time

The optimal time range between transfection and assay for gene activity varies with cell type, promoter activity, expression product, etc. Transfection efficiency can be monitored after 24 - 96 h by analyzing the gene product. Reporter genes such as GFP, secreted alkaline phosphatase or luciferase can be used to quantitatively measure gene expression.

Additional products for your transfection experiments

- **Lullaby Stem** - a transfection reagent dedicated to siRNA transfection into stem cells
- **Senescence Assay Kit** - to detect senescence of your stem cells

Purchaser Notification

Limited License

The purchase of this product grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended for in-house research only by the buyer. Such use is limited to the purposes described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us. Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s). This document covers entirely the terms of the research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

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www.bocascientific.com
(781) 686-1631
info@bocascientific.com

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