Protocol

RNA Transfection using the Stemfect RNA Transfection Kit

OVERVIEW

The Stemfect™ RNA Transfection Kit is a proprietary formulation developed specifically for the transfection of mRNA and siRNA into eukaryotic cells. This protocol describes the transfection of mRNA into fibroblast or human embryonic stem (hES) cells (pg 2), the transfection of siRNA into hES cells (pg 4), and the reverse transfection of siRNA into fibroblast cells (pg 6).

Transfection efficiency can vary greatly depending on a number of factors such as cell type, media, culture format, amount of RNA, ratio of Stemfect RNA Transfection Reagent to RNA, and RNA identity and purity. This protocol should be used as a starting point for optimization of the amounts of RNA and transfection reagent. The weight to volume ratio of Stemfect RNA Transfection Reagent (in µl) should be varied from 2 to 6 µl per microgram of RNA. Additionally, if transfecting more than one well, a master mix can be made by multiplying the single well amounts by the number of wells to be transfected.

Product Description	Cat. No.	Format
Stemfect RNA Transfection Reagent Kit	00-0069	1 Kit
Components	**Size**	**Storage**
Stemfect RNA Transfection Reagent	750 µl	4°C
Stemfect Transfection Buffer	30 ml	4°C

ADDITIONAL MATERIALS REQUIRED

- 6-well tissue culture plates
- 24-well tissue culture plates
- 1.5 ml microcentrifuge tubes (sterile, RNase and DNase free)

MATERIAL PREPARATION

- **Stemfect RNA Transfection Reagent**

Equilibrate the vial of Stemfect RNA Transfection Reagent to room temperature. Flick or briefly vortex the tube to mix the solution prior to transfection. Cap the vial quickly after each use to avoid evaporation. Store remaining Stemfect RNA Transfection Reagent at 4°C.

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mRNA TRANSFECTION PROTOCOL

The following procedure is for the transfection of fibroblast or hES cells with mRNA in one well of a 6-well plate. For scaling the procedure up or down, see the amounts in Table 1.

Transfection efficiency and expression of mRNA can be influenced by the structure of the transfected mRNA molecule as well as the cell type being transfected. Typically, eukaryotic mRNA contains a 5'-cap and a 3' poly-A tail. Both the cap and poly-A tail enhance mRNA stability. In addition, specific mRNA sequences can enhance recruitment of the translation machinery and increase protein expression. However, single-stranded RNA can also illicit an immune response in cells leading to interferon-α production and, in turn, lower transfection efficiency and higher cytotoxicity. This response can be modulated through the use of modified nucleotides and media additives such as B18R protein [Alcamí, A., Symons, J.A., Smith, G.L. (2000) The vaccinia virus soluble alpha/beta interferon (IFN) receptor binds to the cell surface and protects cells from the antiviral effects of IFN. Journal of Virology 74 (23): 11230-11239].

1. Plate the cells in a 6-well tissue culture plate at a density that will yield a 50 to 90% confluent culture after 24 to 48 hours.
2. Aspirate the media and add 2 ml of fresh media to the cells 1 to 2 hours prior to transfection and incubate at 37°C and 5% CO_2.
3. Warm the Stemfect RNA Transfection Reagent and the Stemfect Transfection Buffer to room temperature, and thaw the mRNA on ice or at 4°C.
   Note: *Thaw mRNA immediately prior to transfection. Repeated freeze/ thaw cycles may compromise the integrity of the mRNA and should be limited. Gloves should always be worn when handling RNA as contamination with nucleases can cause degradation.*
4. Add 60 µl per tube of the Stemfect Transfection Buffer into 2 sterilized 1.5 ml microcentrifuge tubes.
5. In the first tube, add 4.0 µl of the Stemfect RNA Transfection Reagent to the buffer and pipet to mix.
6. In the second tube, add 1.0 µg of mRNA to the buffer and pipet to mix.
7. Add the diluted transfection reagent solution to the diluted mRNA solution. Pipet to mix.
8. Incubate the mRNA transfection complex for 10 to 15 minutes at room temperature.
9. Add the entire mRNA transfection complex to the cells dropwise using a pipet and gently rock the plate to ensure even distribution within the well.
10. Incubate the cells at 37°C and 5% CO_2.

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Note: *It is not necessary to change the media after transfection unless toxicity is observed. In this case, changing the media after 4 hours can help to alleviate toxicity.*

11. Assess the transfection efficiency 12 to 24 hours post transfection.

Table 1. Suggested Amounts per Well for Scaling Transfections with mRNA

Culture vessel	Surface area/well	Growth Medium	mRNA	mRNA Dilution Volume	Stemfect RNA Transfection Reagent	Stemfect RNA Transfection Reagent Dilution Volume
96-well plate	0.3 cm^2	0.15 ml	0.04 µg	7.5 µl	0.16 µl	7.5 µl
24-well plate	2 cm^2	0.50 ml	0.25 µg	12.5 µl	1.0 µl	12.5 µl
12-well plate	3.8 cm^2	1.0 ml	0.50 µg	25 µl	2.0 µl	25 µl
6-well plate	9.6 cm^2	2 ml	1.0 µg	60 µl	4.0 µl	60 µl
35 mm dish	12 cm^2	3 ml	1.5 µg	75 µl	6.0 µl	75 µl
60 mm dish	20 cm^2	6 ml	2.5 µg	120 µl	10 µl	120 µl
10 cm dish	59 cm^2	15 ml	7.5 µg	360 µl	30 µl	360 µl

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siRNA TRANSFECTION PROTOCOL

The following procedure is for the transfection of hES cells with siRNA in one well of a 6-well tissue culture plate. For scaling the procedure up or down, see the amounts in Table 2.

1. Plate cells in a 6-well tissue culture plate at a density that will yield a 50 to 90% confluent culture after 24 to 48 hours.
2. Aspirate the media and add 2 ml of fresh media to the cells 1 to 2 hours prior to transfection and incubate at 37°C and 5% CO_2.
3. Warm the Stemfect RNA Transfection Reagent and the Stemfect Transfection Buffer to room temperature, and thaw siRNA on ice or at 4°C.
   Note: *Thaw siRNA immediately prior to transfection. Repeated freeze/ thaw cycles may compromise the integrity of the siRNA and should be limited. Gloves should always be worn when handling RNA as contamination with nucleases can cause degradation.*
4. Add 60 µl per tube of Stemfect Transfection Buffer into 2 sterilized 1.5 ml microcentrifuge tubes.
5. In the first tube, add 2.7 µl of the Stemfect RNA Transfection Reagent to the buffer and pipet to mix.
6. In the second tube, add 50 pmole of siRNA to the buffer and pipet to mix.
7. Add the diluted transfection reagent solution to the diluted siRNA solution. Pipet to mix.
8. Incubate the siRNA transfection complex solution for 10 to 15 minutes at room temperature.
9. Add the entire siRNA transfection complex solution to the cells dropwise using a pipet and gently rock the plate to ensure even distribution within the well. The final concentration of siRNA should be approximately 20 nM.
10. Incubate the cells at 37°C and 5% CO_2.
    Note: *It is not necessary to change the media after transfection unless toxicity is observed. In this case, changing the media after 4 hours can help to alleviate toxicity.*
11. Continue incubating the cells, changing media every 24 to 48 hours.
12. Assess the transfection efficiency 24 to 72 hours post transfection.

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Table 2. Suggested Amounts per Well for Scaling Transfections with siRNA						
Culture vessel	**Surface area/well**	**Growth Medium**	**siRNA**	**siRNA Dilution Volume**	**Stemfect RNA Transfection Reagent**	**Stemfect RNA Transfection Reagent Dilution Volume**
96-well plate	0.3 cm^2	0.15 ml	1.5 pmole	7.5 µl	0.08 µl	7.5 µl
24-well plate	2 cm^2	0.50 ml	10 pmole	12.5 µl	0.52 µl	12.5 µl
12-well plate	3.8 cm^2	1.0 ml	20 pmole	25 µl	1.0 µl	25 µl
6-well plate	9.6 cm^2	2 ml	50 pmole	60 µl	2.7 µl	60 µl
35 mm dish	12 cm^2	3 ml	60 pmole	75 µl	3.0 µl	75 µl
60 mm dish	20 cm^2	6 ml	105 pmole	120 µl	5.2 µl	120 µl
10 cm dish	59 cm^2	15 ml	300 pmole	360 µl	15 µl	360 µl

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REVERSE TRANSFECTION PROTOCOL

The Stemfect RNA Transfection Kit can also be used in reverse transfection protocols. In these procedures, the RNA transfection complex is formed in the supplied buffer and incubated with cells during passaging. This method of transfection can save time and is often used in high throughput applications. The following is a general procedure that describes a reverse transfection of human fibroblast cells with siRNA in one well of a 24-well tissue culture plate. For other plate formats or types of RNA, refer to Tables 1 and 2.

1. Warm the Stemfect RNA Transfection Reagent and the Stemfect Transfection Buffer to room temperature, and thaw siRNA on ice or at 4°C
   Note: *Thaw siRNA immediately prior to transfection. Repeated freeze/ thaw cycles may compromise the integrity of the siRNA and should be limited. Gloves should always be worn when handling RNA as contamination with nucleases can cause degradation.*
2. Passage the cells using a robust procedure for the cell type of choice.
3. Dilute the cells in media to a concentration that will yield approximately 50% confluency 24 hours after plating.
4. Add 12.5 µl per tube of Stemfect Buffer into 2 sterilized 1.5 ml microcentrifuge tubes.
5. In the first tube, add 0.52 µl of the Stemfect RNA Transfection Reagent to the buffer and pipet to mix.
6. In the second tube, add 10 pmole of siRNA to the buffer and pipet to mix.
7. Add the diluted transfection reagent solution to the diluted siRNA solution. Pipet to mix.
8. Incubate the siRNA transfection complex for 10 to 15 minutes at room temperature.
9. Add 0.5 ml of the cell suspension to the transfection complex and pipet to ensure complete mixing.
10. Add the entire contents of the tube to one well of a 24-well tissue culture plate.
11. Incubate the cells at 37°C and 5% CO_2, changing media every 24 to 48 hours.
12. Assess the transfection efficiency 24 to 72 hours post transfection.

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