

Protocol

Transfection of Human ES Cells using the Stemfect Enhanced hESC DNA Transfection Kit

Stemfect™ Enhanced hESC DNA Transfection Kit
Cat. No. 00-0063

OVERVIEW

The following procedure was developed using H1 and HUES16 human embryonic stem (hES) cells cultured under feeder-free conditions with NutriStem Medium. Other culture conditions, including growing hES cells on MEF feeder layers, can be used. Transfection efficiency can vary greatly depending on a number of factors such as media, culture format, amount of DNA and reagent, and plasmid identity and purity. This protocol should be used as a starting point for optimization of the amounts and ratio of DNA and Stemfect Polymer. The amounts listed below are for the transfection of one well of a 12-well plate. For other plate formats, see the suggested amounts in Table 1. 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.

Enhanced hESC DNA Transfection Materials			
Name	Part No.	Size	Storage
Stemfect™ 3.0 hESC DNA Transfection Polymer	02-0004	3 x 100 µl	-20°C
Stemfect™ DNA Plasmid Transfection Buffer	02-0002	30 ml	4°C
Stemolecule™ Y27632	04-0012	2 mg	-20°C

ADDITIONAL MATERIALS REQUIRED

- Matrigel™ hESC-Qualified Matrix (BD Cat. No. 354277)
- 12-well Tissue Culture Plates
- 1.5 ml Eppendorf Tubes
- DMSO

MATERIAL PREPARATION

- **Preparation of Y27632:** Quick spin the vial of Stemolecule™ Y27632. Reconstitute with 624 µl of DMSO to make a 10 mM stock solution. Aliquot into appropriate working volumes and store at -20°C. Reconstituted Y27632 can be stored at -20°C for up to 6 months.
- **Preparation of Stemfect Polymer:** Thaw one vial of the Stemfect™ 3.0 hESC DNA Transfection Polymer and dry any condensate before opening. Flick the tube several times to mix. Aliquot into appropriate working volumes and store at -20°C for up to 12 months. Multiple freeze/thaw cycles are not recommended.



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- **Preparation of Matrigel-Coated Plates:** Coat a 12-well tissue culture plate with Matrigel according to the manufacturer's instructions. If plates have been stored at 4°C, move to room temperature 1 hour prior to use.
- **Preparation of Supplemented Medium:** Dilute the 10 mM stock solution of Y27632 in an appropriate amount of cell culture medium to make a 10 μ M working solution. Use Supplemented Medium immediately.

TRANSFECTION PROCEDURE

1. Seed hES cells on one well of a 12-well Matrigel-Coated Plate using Supplemented Medium. The split ratio will vary depending on the type of cell used but generally ranges from 1:4 to 1:6.

Note: *If seeding cells with Y27632 is not desired, cells may be incubated with Supplemented Medium 1 to 4 hours prior to transfection. For information regarding the use of Y27632 in stem cell culture, please see the references below.*

2. Incubate the cells at 37°C and 5% CO₂ for 24 to 48 hrs. Cells should be approximately 70% confluent at the time of transfection.

Note: *A healthy cell culture is imperative for good transfection results. hES cells can be split directly from a feeder layer onto Matrigel or passaged from Matrigel culture with equivalent results.*

3. Prepare Stemfect Polymer, and DNA (see Material Preparation).
4. Add 50 μ l of Stemfect Buffer into two 1.5 ml eppendorf tubes.
5. In the first tube, add 0.67 μ l of the Stemfect Polymer to the buffer and pipet to mix.
6. In a second tube, dilute 6.7 μ g of DNA to the buffer and pipet to mix.
7. Add the diluted Stemfect Polymer solution to the diluted DNA solution.
8. Vortex the resulting Transfection Solution on high for 10 seconds.

Note: *Vortexing for 10 seconds is very important. If the mixture is not sufficiently vortexed, the DNA-Polymer complex may not form completely.*

9. Incubate the solution for 10 minutes at room temperature.
10. In a new 1.5 ml eppendorf tube, add 1.3 ml of Supplemented Medium.
11. After the 10 minute incubation, add the entire Transfection Solution to the tube containing the Supplemented Medium.
12. Aspirate the medium from the cells and add the Transfection Solution that has been diluted in Supplemented Medium.
13. Incubate the cells for 4 hours at 37°C and 5% CO₂.
14. After 4 hours, aspirate the Transfection Solution and add 1 ml of Supplemented Medium.
15. Incubate overnight at 37°C and 5% CO₂.
16. Assess the transfection efficiency 24 to 48 hours post transfection.

Note: *If culturing the cells for longer than 24 hours post transfection, Y27632 should be removed from the culture during subsequent medium changes.*



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Table 1. Suggested Amounts per well for Scaling Stemfect Transfections

Culture vessel	Surface area/ well	Growth Medium	DNA	DNA Dilution Volume	Polymer	Polymer Dilution Volume
24-well plate	2 cm ²	750 µl	3.2 µg	25 µl	0.33 µl	25 µl
12-well plate	3.8 cm ²	1.3 ml	6.7 µg	50 µl	0.67 µl	50 µl
6-well plate	9.6 cm ²	2 ml	13 µg	100 µl	1.3 µl	100 µl
10-cm dish	57 cm ²	10 ml	77 µg	500 µl	7.7 µl	500 µl

REFERENCES

1. Ishizaki, T., Uehata, M., Tamechika, I., Keel, J., Nonomura, K., Maekawa, M., and Narumiya, S. (2000). Pharmacological properties of Y-27632, a specific inhibitor of rho-associated kinases. *Mol Pharmacol* 57: 976-983.
2. Watanabe, K., Ueno, M., Kamiya, D., Nishiyama, A., Matsumura, M., Wataya, T., Takahashi, J.B., Nishikawa, S., Nishikawa, S., Muguruma, and K., Sasai, Y. (2007). A ROCK inhibitor permits survival of dissociated human embryonic stem cells. *Nat Biotechnol* 25: 681-686.

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