SFE0911-1
For reserch use only

SugarFect®

Gene Transfection Reagent for Stem cells & Primary Cells

SF-P50 …… SugarFect P50 (1.0mL)

INTRODUCTION

SugarFect is a non-viral vector made of cationized polysaccharide for gene transfection of eukaryotic cells. This reagent uses sugar receptor-mediated endocytosis to deliver a desired nucleic acid (DNA) into a target cell. SugarFect has shown low cytotoxicity and some good results in primacy cells and stem cells.

For users requires extremely low cytotoxicity, please try our original "**Subfection**" method. **Subfection** is a one of the reverse-transfection method which uses cell adhesion protein and original coating solution for cell culture plate (see 2. Subfection Protocol).

PROCEDURE

The following protocol is written for transient transfection of plasmid DNA into adherent eukaryotic cells.

► Mark denotes procedures written for transfection of adherent eukaryotic cells in a 12-well plate.

Additional Materials Required, but Not Supplied

- Sterilized double distilled water **(DDW)**
- Dulbecco's Phosphate Buffered Saline (-) **(DPBS)**
- Plasmid DNA **(pDNA)** (100 µg/ml) dissolved in DPBS (*1)
- Sterile microcentrifuge tubes
- 12-well culture plate
- Culture medium
- (For 1. Standard protocol)
  Serum & antibiotics-free culture medium
- (For 2. Subfection® protocol)
  Cell adhesion protein (1mg/ml) (pronectin or fibronectin)

(*1) TE buffer is not recommended.

1. Standard Protocol

One Day before Transfection

1. Count and plate enough cells to achieve roughly 80% confluency at the time of transfection. Incubate cells at normal culture condition (e.g. 37°C in a CO_2 incubator) for 24 hours.

► Plate 1.25 x 10^5cells in 1mL of culture medium per well in a 12-well plate.

The Day of Transfection

1. Dilute SugarFect reagent to desired concentration (refer to the table 1 "N/P Ratio" in the Appendix section) using sterilized DDW.

2. Prepare SugarFect-DNA complexes by adding diluted SugarFect reagent drop-by- drop to pDNA at 1:1 ratio (v/v) in a sterile tube.

IMPORTANT: Always add diluted SugarFect reagent drop-by-drop to diluted pDNA. Reversing the order of addition could reduce the transfection efficiency.

3. Mix gently by tapping the tube containing the complexes. Incubate at room temperature without agitation for the minimum of 15 minutes (up to 1 hour) to allow formation of complexes.

► Appropriate amount of SugarFect-DNA Complexes is 50µL per well in a 12-well plate.

4. Change cell culture medium to serum-free medium while waiting for SugarFect-DNA complexes to form.

►Wash cells once with 500µL of DPBS(-) and add 500 µL of serum-free medium per well, return the plate to 37 °C incubator if SugarFect-DNA complexes need more incubation time.

5. Add appropriate amount of SugarFect-DNA complexes to cells in Serum-free medium. Incubate cells with complexes for 3-6 hours under normal culture condition.

►Add 50 µL of SugarFect-DNA complexes to each well.

6. Change medium back to normal culture medium.

►Carefully aspirate serum-free medium and wash with 500µL of DPBS(-) twice. Add 1mL of appropriate culture medium.

7. Incubate the cells at normal culture condition until the assay for gene expression is to be performed.

2. Subfection Protocol

Transfection can be performed in the presence of serum.

The Day of Transfection

1. Prepare coating solution containing cell adhesion protein in sterile tube.

►Dilute 20µL of Reagent A, 40µL of Cell adhesion protein (1mg/ml) to 400µL using DPBS(-).

2. Coat cell culture plate with coating solution, and incubate at 37°C for 1.5hours to allow them to absorb. Use enough volume of coating solution to prevent from drying.

► Add 400µL of coating solution to each well.

3. Prepare SugarFect-DNA complexes by adding diluted SugarFect reagent drop-by- drop to pDNA at 1:1 ratio (v/v) in a sterile tube.

IMPORTANT: Always add diluted SugarFect reagent drop-by-drop to diluted pDNA. Reversing the order of addition could reduce the transfection efficiency.

4. Mix gently by tapping the tube containing the complexes. Incubate at room temperature without agitation for the minimum of 15 minutes (up to 1 hour) to allow formation of complexes.

► Appropriate amount of SugarFect-DNA Complexes is 50µL per well in a 12-well plate.

5. Coat cell culture plate with SugarFect-DNA Complexes, and incubate at 37°C for 30 minutes to allow them to absorb. Use enough volume of coating solution to prevent from drying.

► Dilute 50µL of SugarFect-DNA complexes to 400µL using 350µL of DPBS(-). Carefully aspirate the coating solution of cell culture plate and wash with 500µL of DPBS(-) once. Add 400µL of SugarFect-DNA complexes solution to each well.

6. Count and plate cells onto the surface of substrate which was coated with the SugarFect-DNA complex.

► Plate 5.0 x 10^4cells in 1ml of culture medium per well in a 12-well plate

7. Incubate the cells at normal culture condition until the assay for gene expression is to be performed.

REFERENCES

Review

Jo J, Tabata Y.

Non-viral gene transfection technologies for genetic engineering of stem cells. Eur J Pharm Biopharm. 2008 68(1):90-104.

Original Paper

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Jo J, et al. Expression profile of plasmid DNA by spermine derivatives of pullulan with different extents of spermine introduced. J Control Release. 2007 118(3):389-98.

Okasora T, et al. Augmented anti-tumor therapy through natural targetability of macrophages genetically engineered by NK4 plasmid DNA. Gene Ther. 2008 15(7):524-30.

Nagane K, et at. Practical induction system for dopamine-producing cells from bone marrow stromal cells using spermine-pullulan-mediated reverse transfection method. Tissue Eng Part A. 2009 15(7):1655-65.

APPENDIX

Dulbecco's Phosphate Buffered Saline (-) (1L)

(Sold as Dulbecco's PBS (-)"*Nissui*" in Japan from Nissui Pharmaceutical Co., Ltd.)

Sodium Chloride……..…….. 8,000 mg
Potassium Chloride ……..…….. 200 mg
Disodium Phosphate (anhydrous)……..... 1,150 mg
Monopotassium Phosphate (anhydrous)…..…. 200 mg

Dissolve above ingredients in 1L (pH=7.35 - 7.55, osmotic pressure: 280±5mOs/kg) of double distilled water. Sterilize by autoclaving at 121 °C for 15 minutes, or by filtration.

Dilution of SugarFect (Table 1)

N/P Ratio (*)	SugarFect	DDW	Total Vol.
1	10 µL	90 µL	100 µL
2	20 µL	80 µL	100 µL
3	30 µL	70 µL	100 µL

* N/P (=[Amino group of SugarFect] / [Phosphate of DNA])
* RatioFor stem cells, recommended N/P ratio is 3

Recommended coating solution and SugarFect-DNA complex volume (Table 2)

Cell Culture Plates	Approx. Growth Area	Coating solution for Subfection			SugarFect-DNA complex		
		ReagentA	Cell adhesion protein (1mg/ml)	PBS(-)	Diluted SugarFect	pDNA (100 µg/mL)	Total (/well)
96 well	0.3 cm^2/well	5 µL	10 µL	105 µL	5 µL	5 µL	10 µL
24 well	1.9 cm^2/well	10 µL	20 µL	170 µL	12.5 µL	12.5 µL	25 µL
12 well	3.8 cm^2/well	20 µL	40 µL	340 µL	25 µL	25 µL	50 µL
6 well	9.5 cm^2/well	40 µL	80 µL	680 µL	50 µL	50 µL	100 µL

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