Sunma

Sofast® Transfection Protocol (in vitro, DNA)

Catalog No.11103 0.3ml (50-100 transfections in 35mm dishes)
Catalog No.11105 0.5 ml (80-165 transfections in 35mm dishes)
Catalog No.11110 1.0 ml (165-330 transfections in 35mm dishes)
Catalog No.11120 2×1.0 ml (330-660 transfections in 35mm dishes)
Catalog No.11140 4×1.0 ml (660-1320 transfections in 35mm dishes)

1. Introduction

Sofast® is new generation cationic polymer gene transfection reagent. It has several unique features necessary for efficient transfection, such as DNA condensation and endosomal release, which can improve gene transfection efficiency. Compared with cationic lipids, Sofast® is very stable, easy to handle and more resistant to serum in cell culture. The above advantages make gene transfection much easier and reproducible. Sofast® are widely used for both primary cell and established cell lines.

2. Package and storage

Sofast® is provided in liquid form at a concentration of 5mg/ml.

Sofast® (5mg/ml) is shipped at room temperature and should be stored at 4 ℃ upon arrival. It is stable for one year at 4 ℃.

3. Transfection protocol (Adherent cells*)

3.1 Cell seeding

To obtain optimal transfection efficiency with Sofast®, the cell density should be 60-80% confluent. In 24- well plate, the optimal cell number is 8×10^4 to2.0×10^5 per well. The cells were seeded at 18-24 hours before gene transfection. However, in most case, similar results could be obtained, if transfection was performed at several hours after cell seeding (after cell attached on the bottom). Table 2 shows the recommended number of cells to be seeded in different cell culture device.

Table 1. Recommended numbers of cells to be seeded in cell culture device

Cell culture devices	Areas of device (mm^2)	Cells number	Final volume of medium in cell culture
96 well plate	50	$1.5\text{-}5.0\times10^4$	100μl
48 well plate	100	$3.0\times10^4\text{-}1.0\times10^5$	200μl
24 well plate	200	$8.0\times10^4\text{-}2.0\times10^5$	500μl
12 well plate	401	$1.6\text{-}4.0\times10^5$	1.0 ml
6 well plate	962	$3.0\text{-}8.0\times10^5$	2.0 ml
35mm dish	962	$3.0\text{-}8.0\times10^5$	2.0 ml

60mm dish	2827	1.0-2.5×10^6	6.0 ml
100mm dish	7854	2.5-6.4×10^6	10.0 ml

3.2 Preparation of complex

3.2.1 Dilute 0.6μg plasmid DNA in 30μl serum-free and antibiotic-free DMEM, mix gently.
Notes: optimal MEM (Invitrogen), PBS buffer or 150mM NaCl can also be used in DNA and transfection reagent dilution.

3.2.2 Dilute 1-2μl Sofast® in 30μl DMEM, mix gently.

3.2.3 Add 30μl Sofast® solutions to DNA solution drop wise with vortex.

Notes: The order of mixing two solutions is very important for gene transfection results. Do not reverse the order.

3.3 Incubate for 15-20 min at room temperature.

3.4 Add 60μl Sofast® /DNA complexes into each well while gently swirling the plate.

3.5 Incubate cell at 37 ℃ in a CO2 incubator. The transfection efficiency of reporter gene could be analysis at 24h-48 hours after adding the complexes.

***Gene transfection in suspension cells**

At 1hour after cell seeding, the transfection reagent/DNA complexes can be added into cells followed transfection assay at 24-48 hours after transfection.

4. Factors affect transfection efficiency

4.1 Amount of Sofast® and DNA used in gene transfection depends on the size of cell culture device. Table 3 shows the recommended transfection reagent and DNA in different cell culture devices. The user can find the optimal condition according to different experiment.

Table 3. Recommended transfection reagent and DNA in different cell culture devices

Cell culture devices	DNA solution		Sofast® solution		Final volume (μl)
	DNA (μg)	Volume of DNA solution (μl)	Sofast® (μl)	Volume of Sofast® solution(μl)	
96 well plate	0.15	7.5	0.2-0.5	7.5	15
48 well plate	0.3	15	0.5-0.9	15	30
24 well plate	0.6	30	1-1.8	30	60
12 well plate	1	50	1-3	50	100
6 well plate	2	100	3-6	100	200
35mm dish	2	100	3-6	100	200
60mm dish	6	300	9-18	300	600
100mm dish	16	800	24-48	800	1600

4.2 Sofast® is not affected by serum during transfection, so Sofast®/DNA complexes can be directly added into complete cell medium. But the buffer for diluting Sofast® and DNA should be serum free,

because Sofast® may bind the protein in serum before making Sofast®/DNA complexes.

4.3 If the cell line is very sensitive, the transfection complexes can be removed at 3-4 hour after adding complexes followed by adding fresh medium containing serum.

4.4 Stable transfection

For stable transfection, 6-well plates or 35mm dishes are recommended to perform gene transfection according to the above protocol. The cells could be selected with experiment device at 24-48 hours after transfection.

5. Troubleshooting

Problems	Comment and suggestion
Low transfection efficiency	1.Use optimal amount of plasmid 2.Use high quality plasmid (OD260/280 >1.8) 3.The density and morphology of cell is optimal 4.Optimize the Sofast®/DNA ratio (w/w from 16:1 to 4:1). 5.Set positive control, such as GFP gene and luciferase gene
Cell toxicity	1.The healthy of cell affect the cytotoxicity 2.The cytotoxicity will increase, if the cell density is not optimal. 3.Dcrease the amount of plasmid, while keep the Sofast® /DNA ratio. 4.Reduce the incubation time for some sensitive cell lines. 5.Check gene product is toxic or not 6.Make sure the plasmid is free of endotoxin.

6. Product Warranty and Satisfaction Guarantee

Sunma guarantees the performance of Sofast® gene transfection reagent in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Sunma will replace it free or refund the purchase price. We reserve the right to charge, alter, or modify any product to enhance its performance and design. If Sunma product does not meet your expectations, simply call our technical service department. We will credit your account or exchange the product – as you wish.

A copy of Sunma terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have any questions about product specifications or performance, please call Sunma service department.