Sunma

Sofast® Transfection Reagent

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)
Store at 4°C. Don't freeze.

1. Introduction

Gene transfection means the delivery and introduction of biologically functional nucleic acid into a cell, by which the nucleic acid retains its function within the cell. The nucleic acid includes DNA (plasmid and linear double strand DNA), antisense oligonucleotide and RNAi (RNA interference). Gene transfection has been widely applied in genomic function studies (gene regulation, gene function, signal transduction and drug screen studies) and gene therapy studies.

Gene carrier is needed for introducing nucleic acid into cells. Both viral and non-viral gene carrier have been used in gene transfer. Viral vectors have highest efficiency, but the safety of virus, the high expense and the complicated procedure in viral vector preparation hampers its applicability. On the other hand, nonviral transfection reagent is needed to deliver viral DNA into cell during viral vector preparation.

Gene transfection reagent is needed to deliver the target gene into a cell during gene transfection. Calcium phosphate shows low transfection efficiency, and is of no effect for a large number of cell lines, so it doesn't meet the most needs in this field. Presently the most popular gene transfection reagent is cationic lipids and cationic polymers. Both of them can overcome the cellular barriers and carry nucleic acid into cell. Cationic lipids show high transfection efficiency in vitro gene delivery. However, they are not suitable for in vivo administration, because they will be rapidly cleared by the plasma, moreover they can accumulate within the lung tissue and induce potent anti-inflammatory activity in vivo, which will induce high level of toxicity. Owing to above limitation of cationic lipid, there is a growing interest in cationic polymer gene carriers.

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, cationic polymers are 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.

1.1 Stable and high transfection efficiency

The stable and high transfection efficiency of Sofast® was compared with some famous commercial transfection reagents. Sofast® is more stable both in presence and absence of serum, Sofast® shows higher transfection efficiency than cationic lipid in some most common used cell lines, and their transfection efficiency are similar in majority cell lines. Especially Sofast® shows higher transfection efficienc in HUV-EC(primary cell), which is insensate to most transfection reagents including cationic lipids. So it is a very good gene transfection reagent.

1.2 Low cytotoxicity

The cell survival rate is over 90%, when experiment is carried in suitable condition, and with recommend Sofast® dosage.

1.3 Simplified transfection procedure, transfection can be finished in half an hour

Sofast® is a serum resistant reagent, the transfection procedure is very simple: the DNA /transfection reagent complexes can be directly added into complete cell medium and waited for transfection assay (Fig. 1). Without the worry about changing medium, this makes user easier to arrange the experiment, the transfection can be finished in half an hour, The whole procedure is simple, time saving and flexible. It was found the cytotoxicity was lower when simplified method was used.

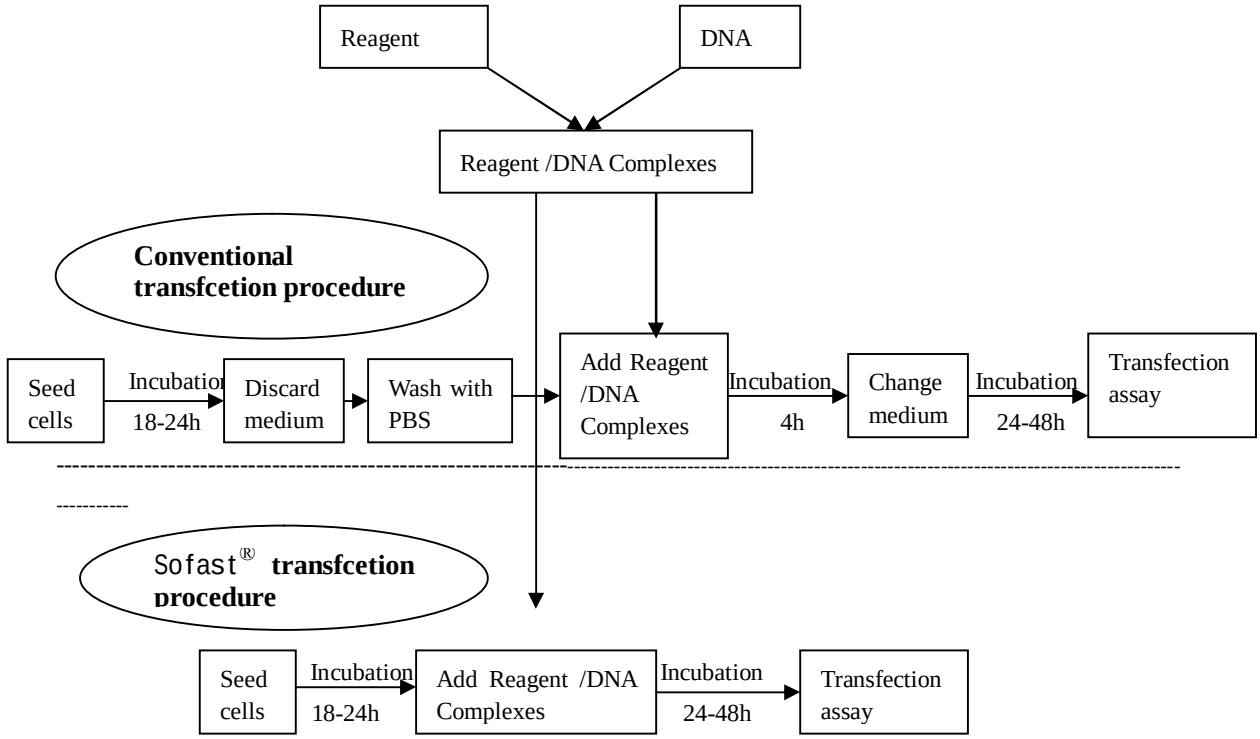

Fig. 1. Comparison of conventional and Sofast® transfcetion procedure

2. Transfection Efficiency

Sofast® is a very potent transfection reagent that can be successfully used in both established cell lines and primary cells. Some cell lines have been successfully transfected with Sofast® are shown in table 1.

Cell line	Origin	Cell type
HEK 293	Human	Embryonic kidney
HeLa	Human	Cervix carcinoma
NIH 3T3	Mouse	Embryo fibroblast

BNL CL2	Mouse	Embryonic kidney cells
HepG2	Human	Hepatocarcinoma
COS7	Monkey	SV40 kidney transformed
CHO	Chinese hamster	Ovary
SHSY-5Y	Human	Neuroblastoma
IMR 32	Human	Neuroblastoma
MRC5	Human	Fetal lung epithelium
MCF7	Human	Breast adenocarcinoma
K562	Human	Chronic leukemia
SKOV-3	Human	Ovarian adenocarcinoma
IGROV-1	Human	Ovarian adenocarcinoma
HUV-EC(primary cell)	Human	Umbilical endothelial
C6 (primary cell)	Rat	Glioma C6
Primary cell	Rat	Neonatal Rat Cardiomyocyte
SGC-7901	human	Gastric cancer
Eca-109	human	Esophageal carcinoma
SK-N-SH	human	Neuroblastoma
ZR-75-1	human	Breast cancer
THP-1	Human	Monocyte
WEHI231	Mouse	Immature B
SK-HEP-1	Human	Liver adenocarcinoma
P815	Mouse	Mastocytoma
BGC-823	Human	Gastric carcinoma
CV-1	African green monkey	Kidney
SiHa	Human	Cervical carcinoma
A549	Human	Lung carcinoma
MDA-MB-231	Human	Breast adenocarcinoma
T-47D	Human	Breast cancer
U-937	Human	Histiocytic lymphoma
HuH-7	Human	Hepatoma
C929	Human	Bbone marrow
XG-1	Human	Myeloma
MC3T3-E1	Rat	Osteogenic
Hep-2	Human	Larynx carcinoma squamous

MDCK	Dog	Kidney (Madin-Darby)
BMSC	Mouse	Bone marrow stromal
BEL-7402	Human	Hhepatic carcinoma
2BS	Human	Diploid fibroblast
U373	Human	Glioblastoma astrocytoma

3. 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 ℃.

4. Transfection protocol (Adherent cells*)

4.1 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 2. 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	$1.0\text{-}2.5\times10^6$	6.0 ml
100mm dish	7854	$2.5\text{-}6.4\times10^6$	10.0 ml

4.2 Preparation of complex

4.2.1 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.

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

4.2.3 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.

4.3 Incubate for 15-20 min at room temperature.

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

4.5 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.

5. Factors affect transfection efficiency

5.1 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

5.2 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.

5.3 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.

5.4 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.

6. 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.

7. 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.