CANFAST Transfection Reagent

1mg/ml

Manual for Cat.nº:

T0082

T0083

Store at 4 °C

PRODUCT MANUAL
Version 3.0
Last updated: August 2013

www.canvaxbiotech.com

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1.MATERIALS PROVIDED, KIT STORAGE AND EXPIRATION DATE

Item	TOO82	T0083
CANFAST transfection reagent (1 µg/µl)	1.5 ml	1 ml
GFP-Plasmid transfection control (1 µg/µl)	15 µl	10 µl

Storage temperature:CANFAST Transfection Reagent is shipped at room temperature and should be stored at 4ºC upon arrival. It is stable for one year at 4ºC.

Store **GFP-Plasmid** transfection control -20ºC

Expiration date: See on the kit label.

IMPORTANT: *Turbidity should appear upon storage, but this does not affect product performance, just vortex the vial before opening.*

2. INTRODUCTION

There are several molecular biology techniques in which a gene transfection reagent is needed to deliver foreign DNA into the nucleus of cells, a process called gene transfection. Plasmid DNA, siRNA, mRNA, oligonucleotides, or even proteins such as antibodies, may be transfected into a cell. Cells not having integrated foreign DNA into the host genome but which is expressed for a limited time, about 24-96 hours, are called transient transfectants, and cells having integrated exogenous DNA in their genome are called stable transfectants. For stable transfection it is also necessary to introduce a gene for drug resistance, thus selecting only transfected cells.There are mainly four methods for introducing foreign DNA and other macromolecules into a cell: 1) methods based on chemical agents such as calcium phosphate, dendrimers, liposomes such as cationic liposomes, and cationic polymers such as DEAE-dextran. Any chemical agents used to transfect cells have to get through the exogenous genetic material, that has a negative charge, through the cell membrane, which is also negatively charged. Calcium phosphate, cationic lipid, DEAE-dextran… can do this. 2) Methods based on direct delivery such as electroporation, sono-poration, microinjection and biolistic particle delivery systems. These methods need a specific system that simply push through the cellular membrane and introduce foreign DNA into the cells. 3) Methods based on biological particles as viral vectors such as lentivirus, baculovirus, adenovirus and retrovirus. These methods require a higher level of safety in handling. 4) Methods based on particles, such as nanoparticles.

The method, based on carrier molecules, is the simplest technique, with many advantages. So far,the most popular gene transfection reagents are cationic lipids and cationic polymers. Both of them can overcome the cellular barriers and carry nucleic acid into the cell.

For maximum efficiency of transfection, it is necessary having cells growing under ideal conditions. On the other hand, DNA must be RNA-free, and free of chemicals, microbial contamination and proteins. It is also important to optimize the transfection protocol to get the desired efficiency because each cell type requires specific transfection conditions. Reporter genes are recommended to optimize transfection.

CANFAST is a 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, inexpensive, easy to handle and more resistant to serum in

cell cultures. The above advantages make gene transfection much easier and reproducible. **CANFAST** is widely used for both primary cell and established cell lines with minimal cytotoxicity and optimal transfection efficiency. **CANFAST** can be used for stable and transient transfection.

3. FEATURES

Low cytotoxicity	The cell survival rate is over 90% when the experiment is carried out in suitable conditions and with the recommended amount **of CANFAST** Transfection Reagent.
Stability in serum	**CANFAST** is a serum-resistant reagent, so the transfection procedure is very simple: the DNA / transfection reagent complexes can be directly added into complete cell medium and assayed for transfection efficiency after 24-72h, without the extra work of changing media.
High-throughput transfection	As the using of **CANFAST** Transfection Reagent does not require change of medium, it is an ideal reagent for high-throughput transfection.
Transfection can be finished in half an hour	The whole procedure is simple, time-saving and flexible.
High transfection efficiency in common cell lines	When compared with some well-known transfection reagents,**CANFAST** is more stable both in presence and absence of serum and shows a higher transfection efficiency than other transfection reagents in many of the most common used cell lines.

4. SUCCESFULLY TRANSFECTED WITH CANFAST

CANFAST Transfection Reagent offers high transfection efficiencies into a wide variety of cell lines, both adherent and suspension cell lines. It is able to transfect with low toxicity various adherent cell types as fibroblasts, embryonic fibroblasts, and epithelial cells. CANFAS Ttransfects successfully mammalian cell lines of different origins with minimal cytotoxicity and maximum transfection efficiency.

CANFAST Transfection Reagent allows transfection of cells growing faster, and slower growing cells. CANFAST Reagent has the advantage that it can be used to transfect cell types that are serum-sensitive, such as primary cell cultures, because this Transfection Reagent can be used in the presence of serum.

Cell line	Origin	Cell type
CHO-K1	Hamster	Chinese ovary cells, epithelial
HEK 293	Human	Embryonic kidney fibroblast
BOSC 23	Human	Kidney; transformed with adenovirus 5 DNA, epithelial
HepG2	Human	Hepatocarcinoma, epithelial
A431	Human	Squamous carcinoma
SW480	Human	Colon adenocarcinoma
56FHT 80	Human	Fetal trachea epithelium cells
6CFSMEo-	Human	Submucosal gland epithelium cells
9HTEo-	Human	Adult trachea epithelial cells
A549	Human	Type II pneumocytes
CACO-2	Human	Colorectal adenocarcinoma cells
CFNPE9o-	Human	Nasal epithelium cells
CFPEo-	Human	Trachea epithelium cells
HCS-2/8	Human	Chondrocyte-like cells
HeLa	Human	Cervix epitheloid carcinoma
Hep 2C	Human	Epidermal carcinoma cells
Jurkat	Human	T cell leukemia
KB	Human	Epithelial cells
MCF7	Human	Breast adenocarcinoma cells
Cos-7	Monkey	Kidney cells
B16F10	Mouse	Skin melanoma, epithelial

Cell line	Origin	Cell type
BW5147	Mouse	AKR/J T cell lymphoma
P815	Mouse	Matocytoma
NIH3T3	Mouse	Embryonic fibroblast
BNL CL.2	Mouse	Hepatocytes
C-26	Mouse	Colon carcinoma cells
C2C12	Mouse	myoblasts
CT26	Mouse	Non-immunogenic colon carcinoma
L929	Mouse	Subcutaneous connective tissue fibroblasts
MCA-38	Mouse	Colon carcinoma cells
Neuro2A	Mouse	Neuroblastoma cells
SH-SY5Y	Human	Neuroblastoma cells
LLC-PK1	Porcine	Kidney epithelial cells
RBL2H3	Rat	Basophilic leukemia

Primary cell cultures	Origin
Postmitotic neurons	Human
Embryonic stem cells	Human
Embryonic stem cells	Mouse
Postmitotic neurons	Rat

5. OPTIMIZATION OF TRANSFECTION EFFICIENCY

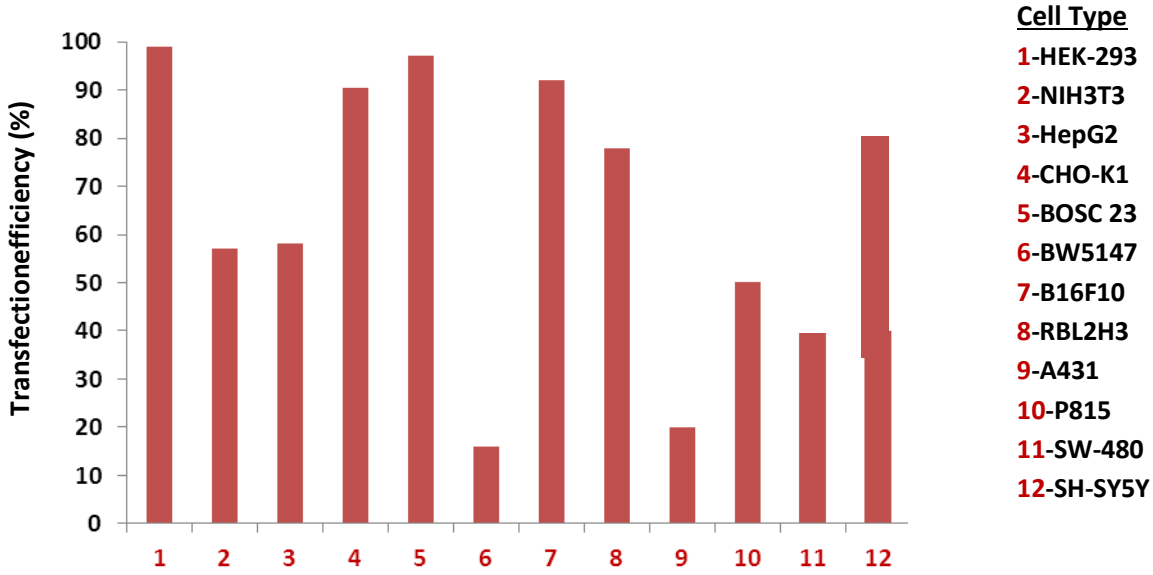


Fig.1- Transfection efficiency in different cell lines using Canfast Transfection Reagent and a green fluorescent protein expression plasmid.

For each cell type it is recommended to optimize specific transfection conditions. Some of the most important factors to be considered are number of cells, amount of DNA, ratio of DNA / **CANFAST**, length of time cells are exposed to the transfection mix, presence or absence of serum for transfection.See some optimization recommended parametersin Tips & Bench of transfection.

It is necessary havingthe cells in exponential-growth phase and in characteristic morphological conditions, and to usequality DNA (free of RNA, nucleases, contaminants and protein). Transfection efficiency varies with the type of cell to be transfected and the transfection conditions used. In your optimization experiments, it is important to have a plasmid with a reporter gene, e.g. green fluorescent protein or a tagged protein with an available antibody. This allows you to check both cells before transfection and the transfection process.

An example of optimisation of the HEK-293 cell line transfection consists in increasing amount of **CANFAST** and constant amount of green fluorescent protein expressing plasmid. When cells are transfected with 6µl of CANFAST, maximum transfection efficiency is obtained. Greater volumes of **CANFAST** have a saturating effect.

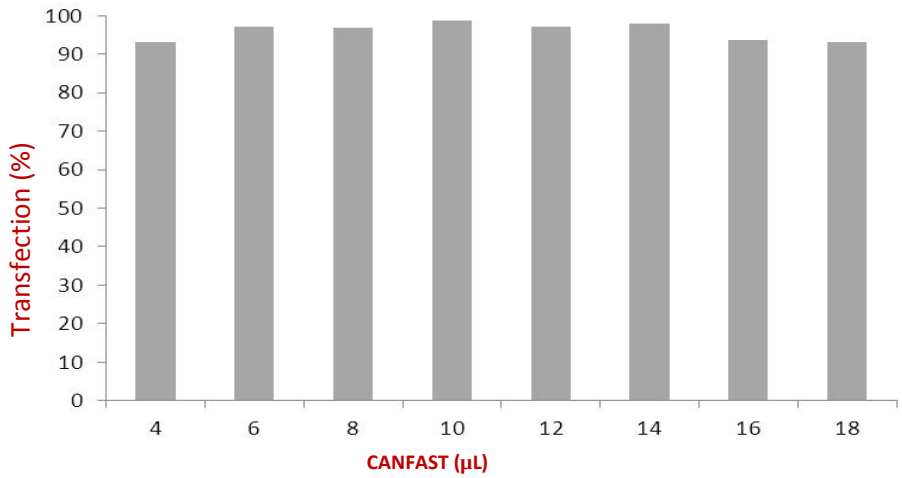


Fig. 2- HEK-293cells were seeded to 3x10^{5}cells/well onto 6 well plates one day before the transfection. Conditions of transfection were 2µg DNA and increasing volumes of CANFAST. Expression plasmid uses cytomegalovirus promoter.

The amount of **CANFAST** Transfection Reagent used in transfection depends on the amount of DNA to be transfected. Graphics below show that in HEK-293, 1 µg plasmid DNA is enough to obtain the highest efficiencies of transfection, using 6µl of **CANFAST**. Higher volumes are saturating and no linear relationship is observed. CHO-K1 cell line is the same. Transfection efficiency varies from a cell line to another. Many factors play also an important role in this process. Thus, it is essential to adjust all these elements to obtain higher transfection efficiency.

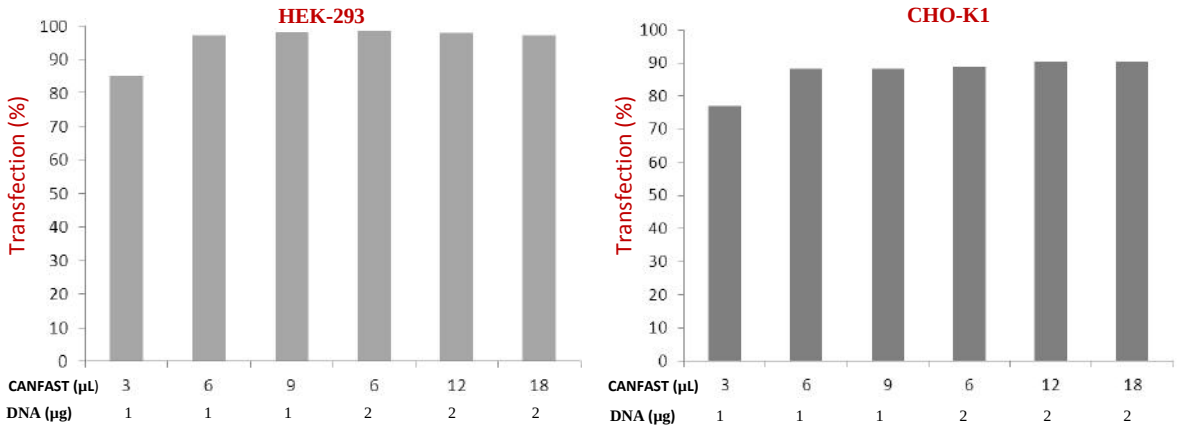


Fig.3-Both HEK-293cells and CHO-K1 were seeded onto 6-well plate one day before the transfection. HEK-293were seeded to 3x10^{5}cells/well while CHO-K1 cells were seeded to 1.5x10^5 cells/ well. Both cell lines were transfected with a green fluorescent protein expression plasmid using two DNA concentrations and six CANFAST Transfection Reagent volumes. Expression plasmid have cytomegalovirus promoter.

6. RECOMMENDED PROTOCOLS

6.1 Transfection Standard Protocol for Adherents Cells (Stable or Transient)

1. Seed the cells

To obtain optimal transfection efficiency with **CANFAST** Transfection Reagent, the recommended confluency for adherent cells is 60-80% the day of transfection. The cells were seeded 18-24 hours before transfection. On the day of transfection it is not necessary to change mediumas **CANFAST** Transfection Reagent is resistant to the serum and, in most cases, to the medium additives present in it. Seed the cells according to the table below.

Recommended Adherent Cells to seed in different growth areas			
Tissue Culture Vessel	**Growth Area (mm^2)**	**Adherent cells to seed 24 h before transfection**	**Final volume of media at the culture (mL)**
96-wellplate	50	$1.5\text{-}5.0\cdot10^4$	0.1
48-wellplate	100	$3.0\cdot10^4\text{-}1.0\cdot10^5$	0.2
24-wellplate	200	$6.0\cdot10^4\text{-}2.0\cdot10^5$	0.5
12-wellplate	401	$1.2\text{-}4\cdot10^5$	1
6-wellplate	962	$2.5\text{-}8.0\cdot10^5$	2
35 mmplate	962	$2.5\text{-}8.0\cdot10^5$	2
60 mmplate	2827	$7.5\cdot10^5\text{-}2.5\cdot10^6$	6
100 mmplate	7854	$1.2\text{-}4\cdot10^6$	10

2. Preparation of CANFAST/ DNA Complex and Transfection.

To prepare the transfection mix, use culture medium without serum because **CANFAST** could react with serum proteins.

a) According to the table below, prepare the DNA solution and the **CANFAST** solution in serum-free medium.

b) Prepare the transfection mix adding **CANFAST** solution dropwise into a DNA solution which is gently being stirred in the Vortex.

c) Incubate the transfection mix for 15-20 minutes, at room temperature.

d) Add the transfection mix dropwise into each well.

e) Gentle shake the plate and incubate it 24 – 72 hours. Some cell lines are more sensitive, it is necessary to change the culture medium to avoid toxicity 1 – 16 hours after adding the transfection mixture.

Recommended Ratios CANFAST Transfection Reagent / DNA					
Tissue Culture Vessel	DNA Solution		CANFAST solution		Transfection Mix (µL)
	DNA (µg)	DNA Solution (µL)	CANFAST (µL)	CANFAST Solution (µL)	
96-well plate	0.15	7.5	0.4-1	7.5	15
48-well plate	0.3	15	1-1.8	15	30
24-well plate	0.6	30	2-4	30	60
12-well plate	1	50	2-6	50	100
6-well plate	1-2	100	6-12	100	200
35 mm plate	1-2	100	6-12	100	200
60 mm plate	3-6	300	18-36	300	600
100 mm plate	8-16	800	48-96	800	1600

6.2 Transfection Standard Protocol for Suspension Cells (Stable or Transient).

1. Seed the cells

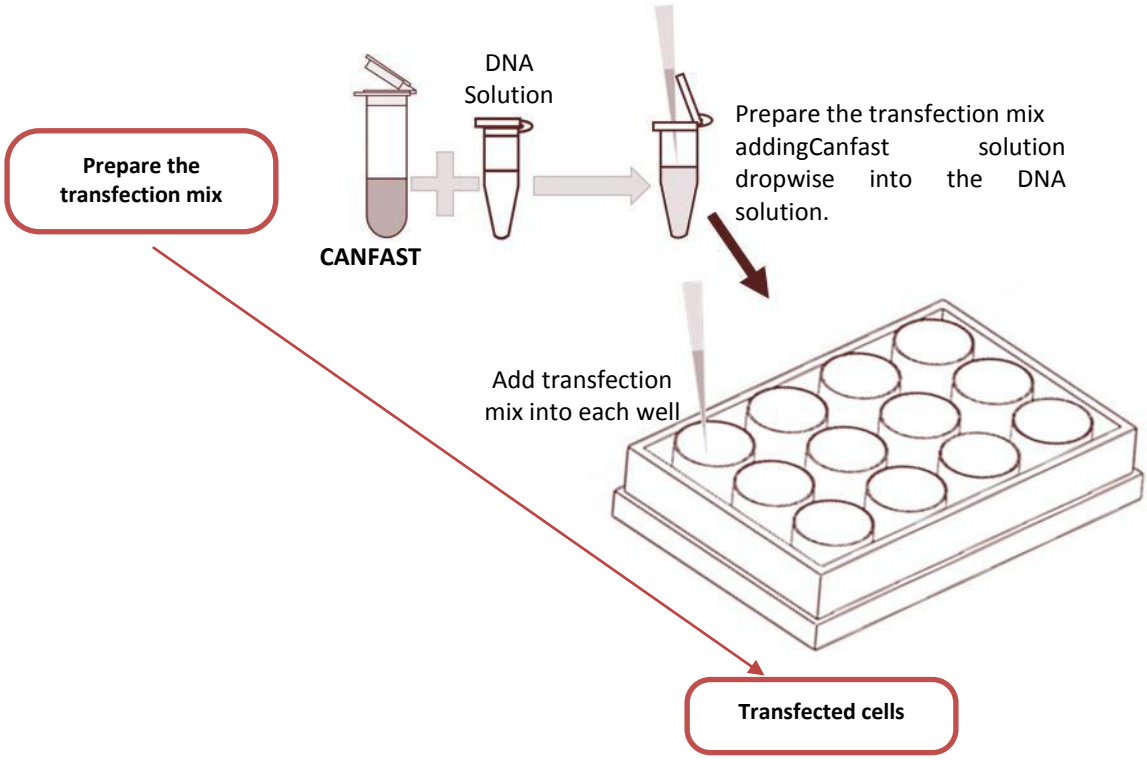
In this case, it is not necessary to seed the cells the previous transfection day. Seed the cells according to the table below. The number of cells to seed depends on cell growth.

Recommended Suspension Cells to seed in different growth areas			
Tissue Culture Vessel	Growth Area (mm^2)	Adherent cells to seed 24 h before transfection	Final volume of media at the culture (mL)
96-well plate	50	$0.5\text{-}2.5\cdot10^4$	0.1
48-well plate	100	$1.0\text{-}5.0\cdot10^4$	0.2
24-well plate	200	$2.0\cdot10^4\text{-}1.0\cdot10^5$	0.5
12-well plate	401	$4.0\cdot10^4\text{-}2.0\cdot10^5$	1
6-well plate	962	$1.0\text{-}5.0\cdot10^5$	2
35 mm plate	962	$1.0\text{-}5.0\cdot10^5$	2
60 mm plate	2827	$3.0\cdot10^5\text{-}1.5\cdot10^6$	6
100 mm plate	7854	$8.5\cdot10^5\text{-}4.0\cdot10^6$	10

2. Preparation of CANFAST/ DNA Complex and Transfection.

Make the **CANFAST** / DNA mixture and transfect as described in the protocol for adherent cells. If it is necessary to replace medium because of toxicity problems, do that after 1 – 16 hours after adding the transfection mixture.

BRIEF PROCEDURE



7. TROUBLESHOOTING

For questions not addressed here, please contact us at www.canvaxbiotech.com or alternatively contact your local Distributor.

Problems	Solutions
Low efficiency of transfection	**1.**Use an optimal DNA amount **2.**Use high quality DNA, the A260/A280 ratio should be at least 1.8 **3.**Use cells with optimal morphological condition and in exponential growth phase **4.**Optimize the ratio of DNA : CANFAST **5.**Verify that promoter of the transfected DNA is recognized by the target cell **6.**Use control reporter gene
Cellular toxicity	**1.**Verify that the cells are not contaminated with *mycoplasma* **2.** Increase the amount of cells to transfection. **3.**Decrease the amount of DNA keeping the amount of CANFAST **4.**Decrease the exposure time of transfection mix with the cells **5.**Check whether the DNA encodes a toxic protein to these cells **6.**Use endotoxin-free DNA
CANFAST Transfection Reagent solution turbidity	**1.**Turbidity should appear upon storage but this does not affect product performance, just vortex the vial before opening.
Transfection not reproducible	**1.**Confluency and phase of growth must be consistent. Check them. **2.**Thaw a new vial of cells

8. TIPS & BENCH

Transfection is the process of deliberately introducing nucleic acids into cells. For maximum efficiency of transfection,culture conditions and the transfection protocol must be optimized including the optimization of the μg DNA / μl transfection reagent ratio, medium used, length of incubation, etc.

Cell culture

To transfect cells it is very important to consider those culture conditions that can influence transfection efficiency, such as cell health, exponential growth, cell passage number and degree of confluency.
Cells to transfect must be inoptimal morphological conditions and it is necessary to verify that they are not contaminated with mycoplasmae or other microbial contaminants. They must be in an exponential growth phase and do not allow the cells to remain confluent for more than 24 hours. Besides, it is necessary to avoid cells havingan excessive number of passages, because it decreases transfection performance.
For stable transfection studies, during selection, the selective medium must be changed several times a week to eliminate dead cells and debris until colonies can be visualized.

Culture Medium

Cells must be grown in an appropriate medium and supplemented with corresponding serum and growth factors. This medium must be fresh.

On the day of transfection, it is crucial to know whether to change the culture medium, depending on the transfection reagent selected, or if the presence of serum and other additives may interfere with the process of DNA release inside the cell.

Transfection mixture is prepared in free-serum culture medium and without additives so that they do not interfere with theformation of the complex DNA-transfection reagent. The addition of antibiotics to media during transfection may result in cell death because cells are more permeable to them.

Transfection Reagent

There are a lot of transfection methods in the market, such as chemical reagents, cationic lipids, physical methods and viral methods. So far, the most popular gene transfection reagents are cationic lipids and cationic polymers, but they have several limitations. One reagent may work well with certain cells, but a second reagent is a better choice when using other cells. On the other hand, any transfection reagents or transfection methods cause cell death. CANFAST transfection reagent is a pure, high quality, new-generation cationic polymer gene transfection reagent non-toxic for cells. It is effective with many different types of cultured cells and makes it easier for the DNA: transfection reagent complex to cross the membrane. CANFAST can be used for transient expression and for long-term studies.

DNA

Nucleic acids need to be of high quality, free of proteins, other contaminating nucleic acids and chemical contamination, i.e, A260/A280 ratio of 1,8-2,0. DNA suspension will be in sterile water or TE buffer to a final concentration of 0.2-1mg/ml. Endotoxin-free DNA is not necessary for thestandard transfection protocol although it is indispensable endofree DNA purification for virus production.

The optimal amount of DNA to transfect cells depends upon the type of DNA and target cell line, and number of cells used. Increasing the amount of DNA does not necessary in better transfection. It is advisable initially to test different amounts of DNA, as recommended by the specific transfection reagent.

Seeding adherent cultures

When the cells are adherent, it is recommended seeding them the day before transfection at a density such that the next day are at least 60-70% confluent. Some cell types have higher toxicity when transfected at low density. It is recommended to usea 24-well plate format to optimize transfection conditions for a particular cell type before initiating a stable transfection.

To transfect cells in different tissue culture formats, vary the amounts of transfection reagent, DNA cells andmedium used in proportion to the relative surface area. Follow the recommendations of the specific transfection reagent.

Drug selection

Before stable transfection, you will need to determine the amount of antibiotic necessary to kill untrasfected cells. This may vary from one cell type to another. You must do a kill curve with different drug concentrations and after a few days to see what the minimum concentration is and at what timecells die. As selection drug Geneticin (G-418), Hygromycin, Puromycin, Zeocin™, and Blasticidin are often used.

Efficiency and Toxicity

24-48h after transfection can analyze the efficiency of transfection. The optimal time interval depends on the cell type and specific transfected genes. It is advisable to observe the state of the cells and see if there are dead cells and changes in morphology. With viability study will know toxicity. Transfected gene products may be toxic. You can use reporter genes to easily monitor transfection efficiency. Reporter genes are luciferase, green fluorescent protein, and β-galactosidase. Check the transfection efficiency using an appropriate assay for the reporter gene.

DNA/ Transfection Reagent ratio

Charge ratios of DNA: Transfection reagent depend upon the type of DNA, transfection reagent, and target cell line. It is advisable to test different ratios of DNA: Transfection reagent. Each ratio may be optimal for certain cell types or applications but not for others.
When the ratio has been optimized, the optimal amount of DNA and Transfection Reagent will vary depending on the culture dish and the number of cells.

Transfection time

The optimal transfection time depends on the transfection reagent, nucleic acid, and cell line used. For optimization, test transfection times from 30 minutes to 4 hours or overnight. There are cells that lose viability with extended time, especially if the transfection is happening in serum-free medium.

Transient or Stable Transfection

For most researches it is sufficient if the transfected genetic material is only transiently expressed, it is usually not integrated into the nuclear genome, so the foreign DNA will be diluted or degraded through mitosis. To accomplish stable transfection a marker gene is necessary, giving the cell some selectable advantage, for example resistance againsta certain antibiotic. When the antibiotic is then added to the transfected cells, only those cells with the DNA integrated into their genomes will be able to proliferate.

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