Convoy™ Transfection Reagent

Catalog No.11103	**0.25ml**	**(40-80 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	**2x1.0 ml**	**(330-660 transfections in 35mm dishes)**
Catalog No.11140	**4x1.0 ml**	**(660-1320 transfections in 35mm dishes)**

Store at 4°C. Do not freeze. Shake gently before use.

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.

Convoy™ 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. Convoy™ 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 Convoy™ was compared with some famous commercial transfection reagents. Convoy™ is more stable both in presence and absence of serum, Convoy™ 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

Convoy™ shows higher transfection efficiency in HUVEC (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 out in suitable condition at recommend Convoy™ dosage.

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

Convoy™ is a serum resistant reagent; the transfection procedure is much simpler than traditional methods: the DNA /transfection reagent complexes can be directly added into complete cell medium to wait for transfection assay completion (Fig. 1). With no need to change and wash cell medium, the 2 step hands-on transfection procedure can be completed in less than 30 minutes. Convoy™ offers an efficient, robust, and easy way to transfect your cells.

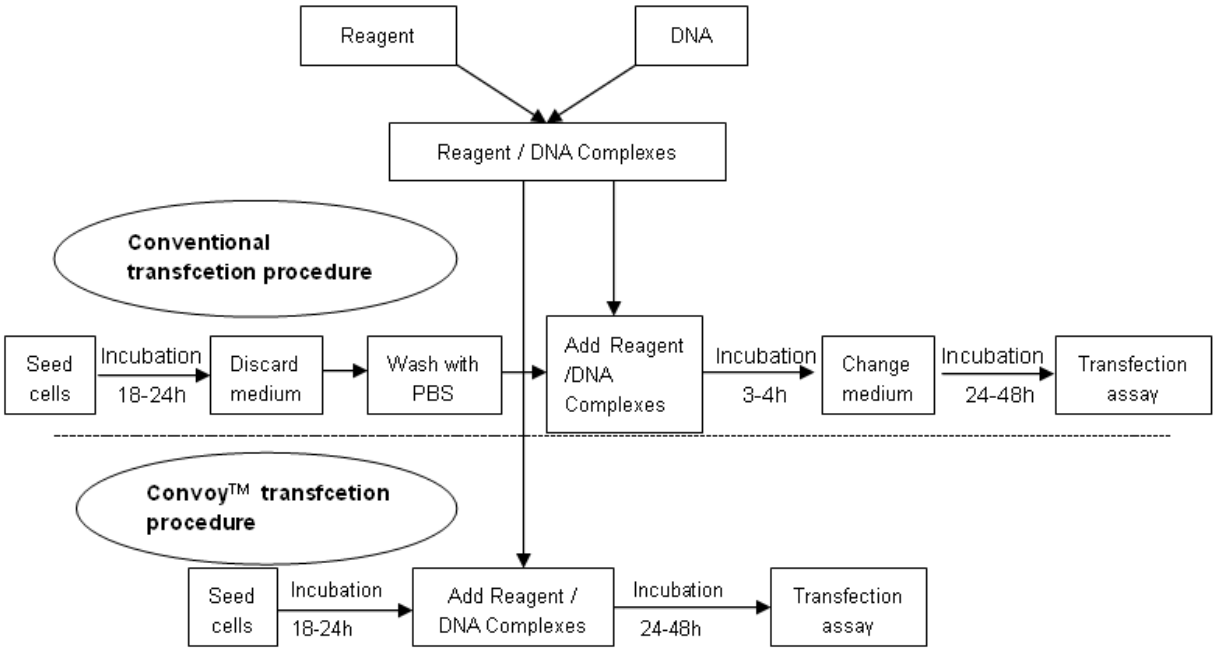

Fig. 1. Comparison of conventional and Convoy™ transfcetion procedure

2. Transfection Efficiency

Convoy™ 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 Convoy™ as shown in Table 1.

Table 1. Cell lines successfully transfected with Convoy™

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
5HSY-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
HUVEC (primary cell)	Human	Umbilical endothelial cell

3. Package and storage

Convoy™ is provided in liquid form at a concentration of 5mg/ml.

Convoy™ (5mg/ml) is shipped at room temperature and should be stored at 4°C upon arrival. It is stable for one year at 4°C. Shake gently before use.

4. Transfection protocol (Adherent cells*)

4.1 Cell seeding

To obtain optimal transfection efficiency with Convoy™, 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 cases, 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	3.0×10^4-1.0×10^5	200μl
24 well plate	**200**	**8.0×10^4-2.0×10^5**	**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 to create **DNA Solution**.
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 Convoy™ in 30μl DMEM, mix gently to create **Convoy™ Solution**.

4.2.3 Add 30μl **Convoy™ Solution**s to 30μl **DNA Solution** and mix with vortex to create **Convoy™ /DNA complexes**.

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 **Convoy™ /DNA complexes** into each well while gently swirling the plate.

4.5 Incubate cell at 37°C in a CO2 incubator. The transfection efficiency of reporter gene could be analyzed 24-48 hours after adding the complexes.

*__Gene transfection in suspension cells__

At 1 hour 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 Convoy™ and DNA used in gene transfection is dependant upon the size of cell culture device.
Table 3 shows the recommended transfection reagent and DNA in different cell culture devices. The user can optimize condition according to different experiment.

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

Cell culture devices	DNA solution		Convoy™ solution		Final volume (μl)
	DNA (μg)	Volume of DNA solution (μl)	Convoy™ (μl)	Volume of Convoy™ 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

Table 4. Recommended transfection reagent and Oligo amount

Amounts oligonucleotide ug	Volume of Convoy™ ul
20-mer	
0.5	1 ~ 1.5
1	2 ~ 3
2	3 ~ 6
4	7 ~ 9
6	10 ~ 14
40-mer	
5	2 ~ 3
10	4 ~ 6
20	8 ~ 10
40	18 ~ 20

5.2 Convoy™ is serum resistant and not affected by serum during transfection, so Convoy™/DNA complexes can be directly added into complete cell medium. But the buffer for diluting Convoy™ and DNA should be serum free, because Convoy™ may bind the protein in serum before making Convoy™/DNA complexes.

5.3 If the cell line is very sensitive, it is recommended that the transfection complexes to 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 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 Convoy™/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 healthiness of cell affect the cytotoxicity. 2. The cytotoxicity will increase, if the cell density is not optimal. 3. Decrease the amount of plasmid, while keep the Convoy™ /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.