

Convoy™ Platinum In Vitro DNA Transfection Reagent (Ver. II)

Store at 4°C

A General Protocol for Transfecting Mammalian Cells

This product is for laboratory research ONLY and not for diagnostic use

Introduction:

Convoy™ Platinum In Vitro DNA Transfection Reagent (Ver. II) is upgraded version of Convoy™ Platinum In Vitro DNA Transfection Reagent. With a new chemistry, more DNA condensing groups were released in the new version compared with old version Convoy™ Platinum, leading to 3~4 times more efficient in DNA delivery. Convoy™ Platinum(Ver.II) was shown to deliver genes to various established cell lines as well as primary cells.

Important Transfection Guidelines for New Version:

- Do NOT follow transfection procedures for Convoy old version. Read protocol for new version carefully before transfection
- For high efficiency, transfect cells at high density. ~90% confluency is highly recommended
- To lower cytotoxicity, transfect cells in presence of serum (10%) and antibiotics
- An advanced protocol is provided for hard-to-transfect cells only if general protocol gives less than 10% efficiency (see back page)

Part I. General Protocol for Transfecting Mammalian Cells:

Step I. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to the optimal 90~95% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30~60 minutes before transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency. For some specific cells, maximal transfection efficiencies are observed in the presence of serum and antibiotics. We recommend using complete serum/antibiotics containing medium initially.

Table 1. Recommended Amounts for Different Culture Vessel Formats

Culture Dish	Transfection Volume (ml)	Plasmid DNA (µg)	Diluent Volume (mL)	Convoy™ Platinum Reagent (µL)
48 well plate	0.3	0.25	2 x 0.015	0.75
12 well plate	0.75	0.75	2 x 0.038	2.25
6-well plate	1.0	1	2 x 0.05	3.0
35 mm dish	1.0	1	2 x 0.05	3.0
60 mm dish	2.8	2.5	2 x 0.10	7.5
10 cm dish	5.0	3 - 4	2 x 0.25	9 - 12
T75 flask	8.0	9 - 18	2 x 0.40	27 - 54
250 ml flask	18	25 - 50	2 x 0.8	75 - 150

Step II. Preparation of Convoy™ Platinum-DNA Complex and Transfection Procedures

For different cell types, the optimal ratio of Convoy™ Platinum (µL):DNA (µg) is around 3:1. We recommend the Convoy™ Platinum (µL):DNA (µg) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency

with invisible cytotoxicity. To ensure the optimal size of Convoy™ Platinum/DNA complex particles, we recommend using serum-free DMEM with High Glucose to dilute DNA and Convoy™ Platinum Reagent.

The following protocol is given for transfection in 24-well plates, refer to Table 1 for transfection in other culture formats. The optimal transfection conditions for a majority of adherent cell lines are given in the standard protocol described below.

- For each well, add 0.5 ml of complete medium with serum and antibiotics freshly 30~60 minutes before transfection.
- For each well, dilute 0.5 µg of DNA into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly to bring drops to bottom of the tube .
- For each well, dilute 1.5 µl of Convoy™ reagent (Ver. II) into 25 µl of serum-free DMEM with High Glucose. Vortex gently and spin down briefly.
- Add the diluted Convoy™ Platinum Reagent immediately to the diluted DNA solution all at once. (Important: do not mix the solutions in the reverse order)
- Vortex- mix the solution immediately and spin down briefly to bring drops to bottom of the tube followed by incubation of 15~20 minutes at room temperature to allow Convoy™ Platinum-DNA complexes to form. Note: Never keep the DNA/Convoy™ Platinum complex longer than 20 minutes
- Add the 50 µl Convoy™ Platinum/ DNA complex drop-wise onto the medium in each well and homogenize the mixture by gently swirling the plate.
- Remove DNA/Convoy™ Platinum complex and replace with complete serum/antibiotics containing medium 12~18 hours post transfection. For sensitive cells, to lower cytotoxicity, remove Convoy™ Platinum/DNA complex and replace with complete medium 5 hours after transfection.
- Check transfection efficiency 24 to 48 hours post transfection.

Storage: Convoy™ Platinum In Vitro DNA Transfection Reagent is stable for up to 12 months at +4 0C. This item shipped at ambient temperature