Chemi-Trans™ Polyplus DNA Transfection Reagent

（Cat #: T00X）

Introduction:

The Chemi-Trans™ Polyplus DNA Transfection Reagent is formulated as a polycationic polymer-based DNA transfection reagent that works by forming a positively charged complex with DNA and could provide a high transfection efficiency in a variety of common adherent and suspension mammalian cells by endocytosis. This reagent is quite suitable for protein production both in HEK293 and CHO cell lines during the industrial bioprocessing.

Advantages:

- Provide high transfection efficiency with less cytotoxicity.
- Compatible for a broad range of cell lines including the most commonly used HEK293 and CHO cells, grown in adherent and suspension cultures system.
- Excellent for protein and reproducible virus production.
- Very affordable.

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Kit Components:

Cat #	Product Name	Volume	Storage
T00X	Chemi-Trans™ Polyplus DNA Transfection Reagent	1 mL	Store at 2~8°C, and stable for up to 6 months after receipt.

Important Guidelines for Transfection:

(1) For maximum transfection efficiency, using serum-free medium (such as Opti-MEM® Reduced Serum Medium) to dilute DNA and the Endotoxin-free plasmid is a must.
(2) While the standard protocols for DNA transfection being given below, optimization is often needed for maximal transfection efficiency.
(3) Make sure the dilution solution be 1/10 of the total growth medium per well.

Standard Protocol for DNA Transfection of Adherent Cells

Step Ⅰ. Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to 70 ~ 80% confluency at the time of transfection. 2% FBS contained complete culture medium without antibiotics is freshly added to each well 30 ~ 60 min before transfection.

Step Ⅱ. DNA Transfection Protocol

Use the following procedure to transfect DNA into mammalian cells in a 6-well format. For other formats, please refer to ***A Guideline for DNA transfection (Table 2)***. All amounts and volumes are given on a per well basis. For each transfection sample, prepare complexes as follows:

a. Dilute 2.0 µg Endotoxin-free plasmid DNA in 200 µL of serum-free Opti-MEM (or other medium without serum), Vortex to mix gently but thoroughly.

b. Add 6 µL Chemi - Trans™ Reagent to the diluted DNA mixture (prepared in Step a.), and mix the transfection complexes by pipetting up and down. Incubate for 10 ~ 20 minutes at room temperature to let transfection complex form well.

c. Add the 200 µL of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

d. Incubate cells at 37°C in a CO_2 incubator for 24 to 72 hours. Then, to measure the gene silencing by qRT-PCR and Western Blotting respectively.

Table 2: A Guideline for DNA transfection per cell culture vessel

Culture Vessel	Growth Medium (mL)	Serum - Free Medium (µL)	DNA (µg)	Chemi - Trans™ Polyplus Reagent (µL)
	Volume used per well			
96-well	0.1	10	0.1	0.15 ~ 0.4
24-well	0.5	50	0.5	0.75 ~ 2.0
12-well	1.0	100	1.0	1.5 ~ 4.0
6-well	2.0	200	2.0	3.75 ~ 10.0

***[1]** We strongly suggest that keep the concentration of plasmid DNA be 0.5 ~ 2.0 µg/µL, and the Endotoxin-free plasmid is extremely important for a successful transfection.*

[2] To obtain a high yield recombinant protein, optimize transfection conditions by plating the cells two days before the transfection to lower cell density in some sensitive types of cell lines, such as HEK-293, HEK293T, NIH/3T3 and COS, etc. Ensure that cells are 60 ~ 80% confluent.

[3] You may perform a rapid 96-well plate transfections by plating cells directly into the transfection complexes. Prepare complexes in the plate and directly add cells drop wise at twice the cell density as in the basic protocol in the complete growth medium. Cells will adhere as usual in the presence of complexes.