

TOOLStrong Transfection Reagent

Cat. no.: TTF-CZ201 / TTF-CZ201-10

Product Size:

TTF-CZ201 1 ml

TTF-CZ201-10 10 x 1 ml

Storage: For long-term storage, the reagent can be stored at -20°C for 1 year. For short-term

storage and frequent use, it can be stored at 4°C for 6 months.

Introduction

TOOLStrong Transfection Reagent is a cationic polymer-based transfection reagent that can be used for DNA transfection with lower toxicity than liposomal or cationic methods. This product has a broad spectrum for most commonly used established cell lines and is an ideal solution for high performance with maximum compatibility.

Protocol

Transfection protocol in a 24-well plate:

- 1. Cell preparation: Cells should be seeded 16–20 hours prior to transfection with a confluency of approximately 70%. The medium should be refreshed 30 minutes before transfection. Usually, using a culture medium with serum does not affect transfection.
- 2. DNA solution: Add 500 ng of plasmid DNA into 25 µL of serum-free medium for 5 minutes.
- 3. TOOLStrong solution: Add 1.5 μ L of TOOLStrong Transfection Reagent into 25 μ L of serum-free medium for 5 minutes. For the guidelines on DNA plasmid and TOOLStrong amounts and ratio, please refer to Table 1. If you wish to increase the transfection efficiency, you can increase the amount of DNA. For the ratio, please refer to Table 2; the DNA/TOOLStrong ratio is 1:3.
- 4. Mix 25 μ L of TOOLStrong solution and 25 μ L of DNA solution together and tip gently. Incubate at room temperature for 5–15 minutes to form a DNA/TOOLStrong complex.
- 5. Add 50 µL of DNA/TOOLStrong Mix solution into a 24-well plate and mix gently.
- 6. The mixture can be removed after 6–48 hours and refilled with culture medium.

Example of the suspension transfection protocol in a 100-mm Petri dish:

1. Trypsinize cells from a culture disk or flask as per the standard tissue culture procedure. Count the cell density and transfer suitable cells (5×10^6) to a 1.5-mL microcentrifuge tube with culture medium. Mark the tube as the "Cell tube."

Note: To prevent readherence of the cells to the tube, perform this step no more than 1 hour prior to transfection.

- 2. DNA Solution: Add 5 μ g of plasmid DNA into 300 μ L of <u>serum-free medium</u> and incubate for 5 minutes. Mark the tube as the "DNA tube."
- 3. TOOLStrong Solution: Add 15 μ L of TOOLStrong Transfection Reagent into 300 μ L of serum-free medium for 5 minutes. Mark the tube as the "Reagent tube."

Note: For guidelines on DNA plasmid and TOOLStrong amounts and ratio, please refer to Table

- 4. After incubation, transfer all of the solution from the DNA tube to the Reagent tube and mix with pipetting gently. Incubate at room temperature for 5–15 minutes to form a DNA/TOOLStrong complex.
- Transfer 600 μL of DNA/TOOLStrong Mix solution into the Cell tube and mix gently. Incubate for 2 minutes.
- 6. Transfer all the mixture from the Cell tube to the 100-mm Petri dish containing culture medium. Note: For the final culture medium volume, please refer to Table 1. In the case of the 100-mm dish, the final volume is 6 mL (which includes 600 μ L of DNA/Reagent mixture and the medium volume from step 1).

Table 1. Cell culture vessel and operation.

Cell culture plate/Dish	Total	DNA S	Solution	TOOLStrong Solution		
	Medium volume	Plasmid	Serum-free medium	TOOLStrong	Serum-free medium	
96 well	100 μL	250 ng	10 μL	0.75 μL	10 μL	
24 well	500 μL	500 ng	25 μL	1.5 μL	25uL	
12 well	700 μL	750 ng	35 μL	2.25 μL	35 μL	
6 well	1 mL	1 μg	50 μL	3 μL	50 μL	
60mm plate	3 mL	2.5 μg	150 μL	7.5 μL	150 μL	
100mm plate	6 mL	5 μg	300 μL	15 μL	300 μL	

^{*} For the preparation of multiwell transfection for the plate master mix, please refer to Appendix I.

Table 2. Cell culture vessel and operation for higher transfection efficiency.

Cell culture plate/Dish	Total	DNA S	Solution	TOOLStrong Solution		
	Medium volume	Plasmid	Serum-free medium	TOOLStrong	Serum-free medium	
96 well	100 μL	250 ng	10 μL	0.75 μL	10 μL	
24 well	500 μL	500 ng	25 μL	1.5 μL	25uL	
12 well	700 μL	750 ng-1ug	35 μL	2.25 μL -3uL	35 μL	
6 well	1 mL	1-2.5 μg	50 μL	3 -7.5μL	50 μL	
60mm plate	3 mL	2.5 -5.5μg	150 μL	7.5 -16.5μL	150 μL	
100mm plate	6 mL	5 -14μg	300 μL	15-42 μL	300 μL	

Table 3.

Dish/Plate	Cell number	Total	DNA	Solution	TOOLStrong Solution		
(Each well)		Medium volume	DNA	Serum-free medium	TOOLStrong	Serum-free medium	
96 well plate	1.5 x 10 ⁴	100 μ1	250 ng	10 μl	0.75 μl	10 µl	
24 well plate	7.5×10^4	500 μ1	500 ng	25 μl	1.5 μl	25 μl	
12 well plate	1.5×10^5	700 µl	750 ng	35 μl	2.25 µl	35 µl	
6 well plate	5 x 10 ⁵	1 ml	1μg	50 μl	3 μl	50 μl	
60 mm dish	2 x 10 ⁶	3 ml	2.5µg	150 µl	7.5 µl	150 μl	
100 mm dish	5 x 10 ⁶	6 ml	5 μg	300 μl	15 μl	300 μl	

Appendix I.

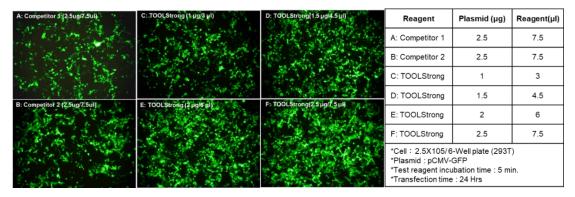
Multiwell transfection for a 96-well plate master mix	Volume required per well	Total volume required if preparing a master mix for a plate
Volume of serum-free medium for transfection complex formation	10 μL	$10 \times 100 = 1,000 \ \mu L = 1 \ mL$ Note: 1 mL for DNA solution and 1 mL for TOOLStrong Solution.
Amount of DNA required per well	0.25 μg	$0.25 \times 100 = 25 \mu\text{g}$
Volume of TOOLStrong Reagent per well	0.75 μL	$0.75 \times 100 = 75 \ \mu L$
Trypsinized cells in complete growth medium	80 μL	$80 \times 100 = 8,000 \ \mu L = 8 \ mL$

Multiwell transfection for a 24-well plate master mix	Volume required per well	Total volume required if preparing a master mix for a plate
Volume of serum-free medium for transfection complex formation	25 μL	$25 \times 25 = 625 \ \mu L$ Note: $625 \ \mu L$ for DNA Solution and $625 \ \mu L$ for TOOLStrong Solution.
Amount of DNA required per well	0.5 μg	$0.5 \times 25 = 12.5 \ \mu g$
Volume of TOOLStrong Reagent per well	1.5 μL	$1.5 \times 25 = 37.5 \ \mu L$
Trypsinized cells in complete growth medium	450 μL	450 × 25 = 11,250 μL = 11.25 mL

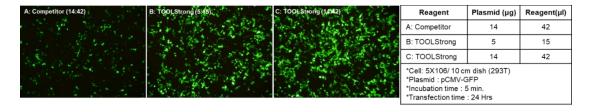
Multiwell transfection for a 12-well plate master mix	Volume required per well	Total volume required if preparing a master mix for a plate
Volume of serum-free medium for transfection complex formation	35 μL	$25 \times 13 = 325 \ \mu L$ Note: $325 \ \mu L$ for DNA Solution and $325 \ \mu L$ for TOOLStrong Solution.
Amount of DNA required per well	0.75 μg	$0.5 \times 13 = 6.5 \ \mu g$
Volume of TOOLStrong Reagent per well	2.25 μL	$2.25 \times 13 = 29.25 \ \mu L$
Trypsinized cells in complete growth medium	630 μL	$630 \times 13 = 8{,}190 \ \mu L = 8.190 \ mL$

Experimental data comparison between TOOLStrong Transfection Reagent and Competitors:

Data 1



Data 2



Suitable for a variety of cell lines:

293T	HepG2	HuH-7	THP-1	K562	A549	BHK-21	HMEC-1	HT-29	Vero
HeLa	HUVEC	CHO-K1	MDCK	C6/36	SP2/1 0	PBMCS	P3X63Ag8	SF9	S2
SKNSH	U118MG								

The product is for research only; not for diagnostic or clinical use.