

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 μL of TOOLStrong Transfection Reagent into 25 μL 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 μL of TOOLStrong solution and 25 μL 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.

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2. DNA Solution: Add 5 µg of plasmid DNA into 300 µL of serum-free medium and incubate for 5 minutes. Mark the tube as the “DNA tube.”
3. TOOLStrong Solution: Add 15 µL of TOOLStrong Transfection Reagent into 300 µL 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.
5. 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 µL of DNA/Reagent mixture and the medium volume from step 1).

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

Table 1. Cell culture vessel and operation.

Cell culture plate/Dish	Total Medium volume	DNA Solution		TOOLStrong Solution	
		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	25 µL
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

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

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

Table 3.

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

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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 μ L	$10 \times 100 = 1,000 \mu\text{L} = 1 \text{ mL}$ Note: 1 mL for DNA solution and 1 mL for TOOLStrong Solution.
Amount of DNA required per well	0.25 μ g	$0.25 \times 100 = 25 \mu\text{g}$
Volume of TOOLStrong Reagent per well	0.75 μ L	$0.75 \times 100 = 75 \mu\text{L}$
Trypsinized cells in complete growth medium	80 μ L	$80 \times 100 = 8,000 \mu\text{L} = 8 \text{ mL}$

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 μ L	$25 \times 25 = 625 \mu\text{L}$ Note: 625 μ L for DNA Solution and 625 μ L for TOOLStrong Solution.
Amount of DNA required per well	0.5 μ g	$0.5 \times 25 = 12.5 \mu\text{g}$
Volume of TOOLStrong Reagent per well	1.5 μ L	$1.5 \times 25 = 37.5 \mu\text{L}$
Trypsinized cells in complete growth medium	450 μ L	$450 \times 25 = 11,250 \mu\text{L} = 11.25 \text{ mL}$

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 μ L	$25 \times 13 = 325 \mu\text{L}$ Note: 325 μ L for DNA Solution and 325 μ L for TOOLStrong Solution.
Amount of DNA required per well	0.75 μ g	$0.5 \times 13 = 6.5 \mu\text{g}$
Volume of TOOLStrong Reagent per well	2.25 μ L	$2.25 \times 13 = 29.25 \mu\text{L}$
Trypsinized cells in complete growth medium	630 μ L	$630 \times 13 = 8,190 \mu\text{L} = 8.190 \text{ mL}$

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Experimental data comparison between TOOLStrong Transfection Reagent and Competitors:

Data 1

			Reagent	Plasmid (μg)	Reagent(μl)
A: Competitor 1 (2.5ug/7.5ul)			A: Competitor 1	2.5	7.5
C: TOOLStrong (1 μg/3 μl)			B: Competitor 2	2.5	7.5
D: TOOLStrong(1.5 μg/4.5 μl)			C: TOOLStrong	1	3
B: Competitor 2 (2.5ug/7.5ul)			D: TOOLStrong	1.5	4.5
E: TOOLStrong (2 μg/6 μl)			E: TOOLStrong	2	6
F: TOOLStrong(2.5 μg/7.5 μl)			F: TOOLStrong	2.5	7.5
			*Cell : 2.5X105/6-Well plate (293T) *Plasmid : pCMV-GFP *Test reagent incubation time : 5 min. *Transfection time : 24 Hrs		

Data 2

			Reagent	Plasmid (μg)	Reagent(μl)
A: Competitor (14:42)			A: Competitor	14	42
B: TOOLStrong (5:15)			B: TOOLStrong	5	15
C: TOOLStrong (14:42)			C: TOOLStrong	14	42
			*Cell: 5X106/ 10 cm dish (293T) *Plasmid : pCMV-GFP *Incubation time : 5 min. *Transfection time : 24 Hrs		

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	SP2/10	PBMCS	P3X63Ag8	SF9	S2
SKNSH	U118MG								

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