TOOLSFect Transfection Reagent

Cat. no. TTM-TF01

Product Size:

Component	Size
TOOLSFect Transfection Reagent	1 mL
Working buffer	50 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

TOOLSFect Transfection Reagent is a cationic polymer-based reagent that deliver DNA into a wide variety of cell lines with high efficiency and low toxicity. The protocol does not require removal of serum or culture medium and does not require washing or changing of medium after introducing the reagent/DNA complex.

Application

- Plasmid transfection
- Co-transfection of plasmids
- Co-transfection for virus production

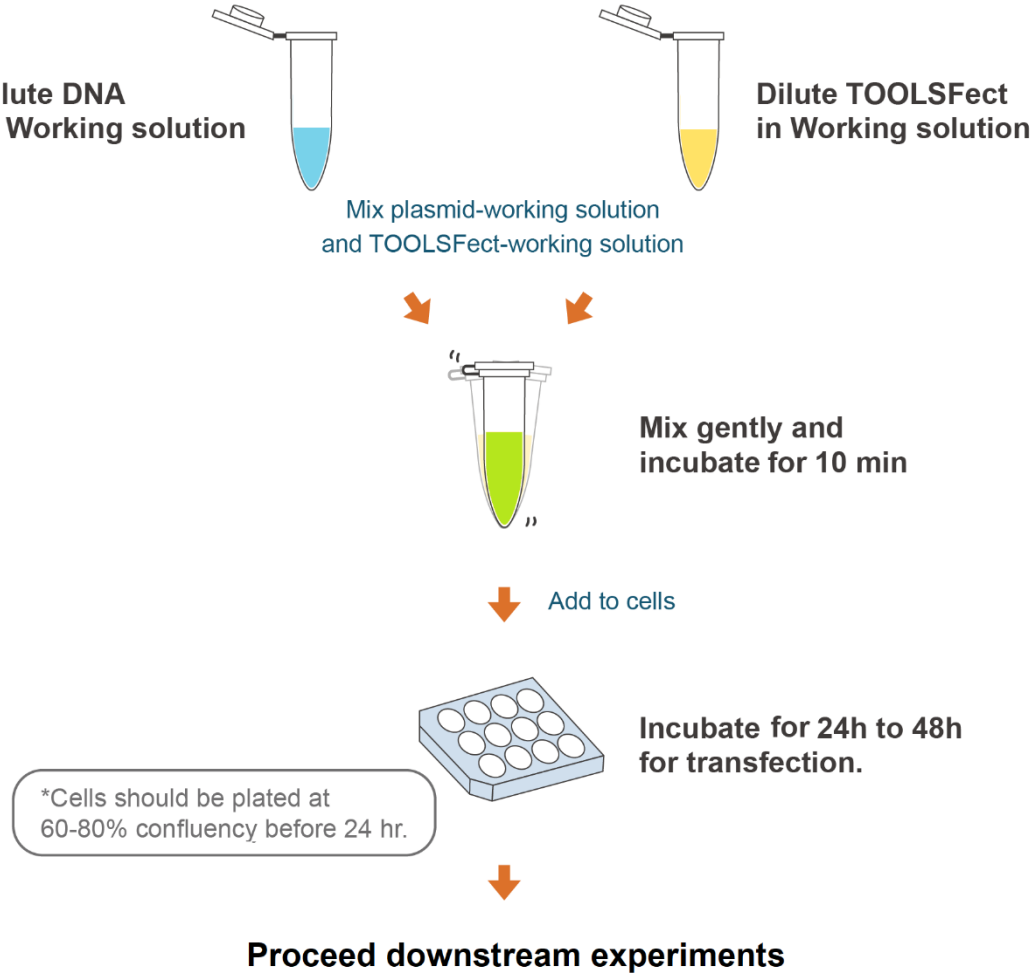
Materials required but not supplied with the product

- Plasmid DNA (0.5 – 5 μg/μL)
- Cell culture medium with serum appropriate for the cell type being transfected
- 96-well or other culture plates

Important Notes

- Transfection complex for use must be prepared with working buffer.
- There is no need to replace or add the medium after transfection.
- The amount of TOOLSFect Transfection reagent required for efficient transfection might very according to cell type and passage times. Testing trial is highly recommended to determine an optimum amount when handling a new cell line.

Workflow


Dilute DNA
in Working solution
Dilute TOOLSFect
in Working solution
Mix plasmid-working solution
and TOOLSFect-working solution
Mix gently and
incubate for 10 min
Add to cells
Incubate for 24h to 48h
for transfection.
*Cells should be plated at
60-80% confluency before 24 hr.
Proceed downstream experiments

Protocol

A. Plating cells

Adherent should be seeded 16–20 hours prior to transfection with a confluency of approximately 60 – 80%. The medium should be refreshed 30 minutes before transfection. Usually, using a culture medium with serum does not affect transfection. Adjust cell numbers proportionately for different size plates (Table 1).

B. Prepare the Transfection complex

1. Dilute TOOLSFect transfection reagent in working buffer (Table 1)
2. Dilute plasmid DNA in working buffer (Table 1)

Table 1. Preparation of complexes for transfection in different cell culture formats.

Component	96-well (0.2 mL)	24-well (0.5 mL)	12-well (1 mL)	6-well (2 mL)
Adherent cells	0.5 - 2 x 10^4	4 - 7 x 10^4	0.8 – 1.5 x 10^5	1.8 - 2.5 x 10^5
Diluted TOOLSFect				
TOOLSFect	0.5 µL	1 µL	3 µL	6 µL
Working buffer	9.5 µL	24 µL	47 µL	94 µL
Total volume	10 µL	25 µL	50 µL	100 µL
Diluted DNA				
DNA amount (0.5 µg/µL stock)	1 µL (0.5 µg)	2 µL (1 µg)	6 µL (3 µg)	12 µL (6 µg)
Working buffer	9 µL	23 µL	44 µL	88 µL
Total volume	10 µL	25 µL	50 µL	100 µL

The following protocol is in the 96 well plate:

3. The ratio of DNA to TOOLSFect transfection reagent is 1:1, dilute 0.5 µg/µL of DNA in working buffer to total volume of 10 µL, dilute 0.5 µL TOOLSFect transfection reagent in working buffer to total volume of 10 µL, Mix diluted DNA solution and diluted TOOLSFect transfection reagent solution, tip gently. Incubate at room temperature for 10 minutes to form a DNA/TOOLSFect transfection complex.

Note: Please spin down the TOOLSFect transfection reagent before use.

4. Add 20 µL DNA/TOOLSFect transfection complex mixture per well into 96-well cell culture and mix gently. Return cells to the incubator for 24–48 hours.
5. Measure transfection efficiency using an assay appropriate for the reporter gene. For transient transfection, cells are typically assayed 24–48 hours after transfection.

Supplementary information

A. Transfection in HUVEC

TOOLSFect transfection reagent and Competitor agents were used to Human umbilical vein endothelial cells (HUVEC) with a GFP-expressing plasmid in a 12-well plate format (1.2×10^5 cells/well), using 3 µg plasmid/well and the recommended protocols for each reagent. GFP expression was analyzed 48 hours post transfection.

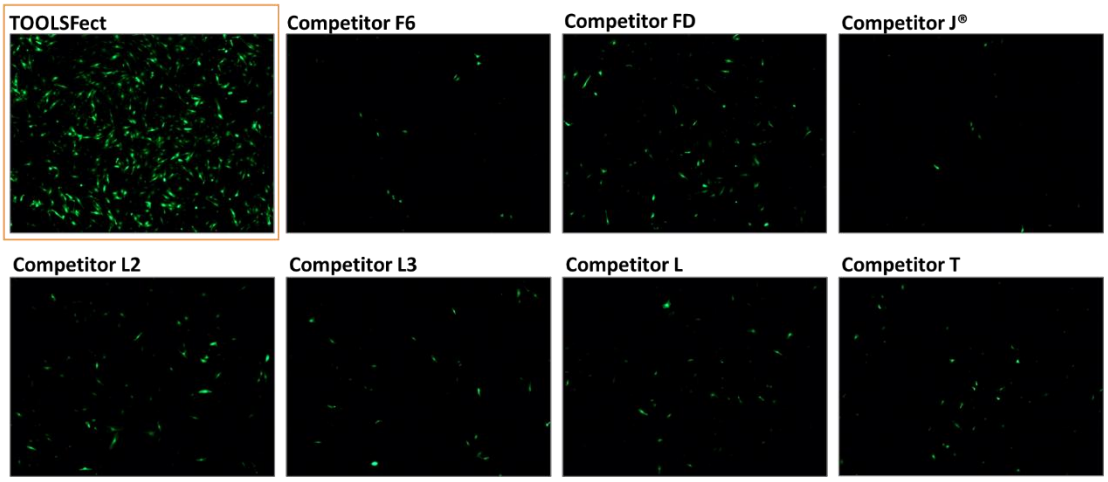


Figure 1. Transfection efficiency in HUVEC.

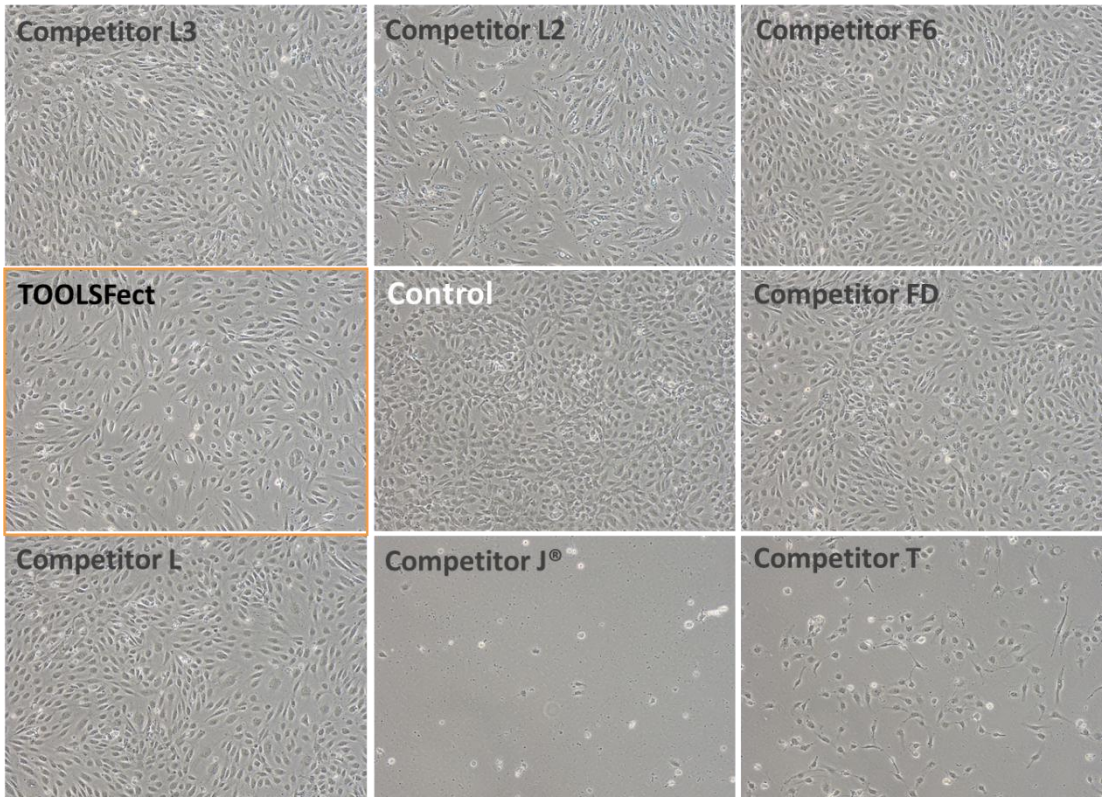


Figure 2. Cell morphology and viability in HUVEC post transfection.

B. Application in Lentivirus production

TOOLSFect transfection reagent and Competitor agents were used to transfect plasmid for lentivirus production, pCMV-ΔR8.91 (12 kb), pMD.G (6.5 kb), and pLAS2w.RFP-C.Pneo (9 kb) into 293T cells in a 6-well plate format. The recommended protocols for each reagent were applied. The assembled lentivirus of RFP was collected and further used to infect the cells and RFP expression was analyzed 72 hours after lentivirus infection

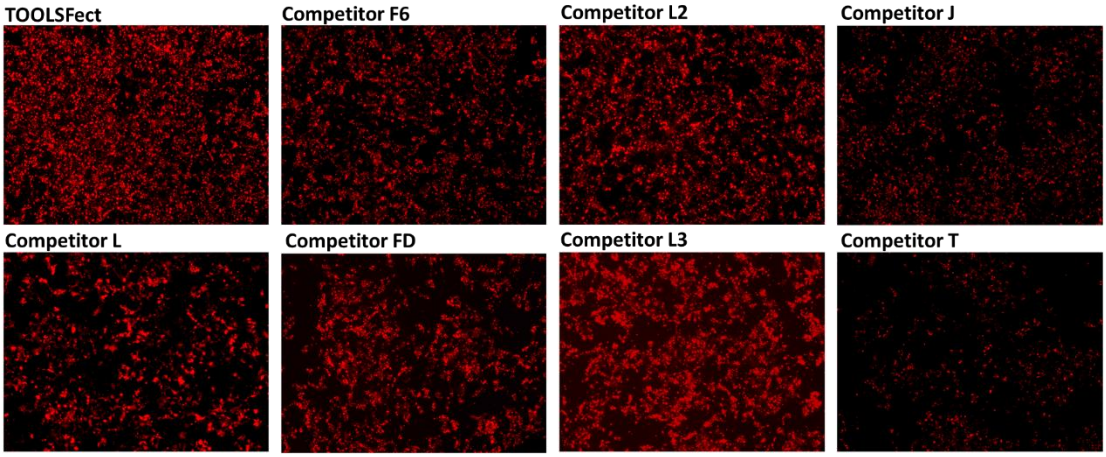


Table 2. Amounts of plasmid DNAs used in Lentivirus production

Component	6-well
pCMV-ΔR8.91 (12 kb)	1.8 µg
pMD.G (6.5 kb)	0.2 µg
pLAS2w.RFP-C.Pneo (9 kb)	2 µg
TOOLSFect	6 µL
293T Cells	2 x 10^5/well
Medium	2 mL

Note: Co-transfection ratio of plasmid DNA : TOOLSFect transfection reagent is recommended to try 1:1, 1:1.5, and 1:2.

C. Transfection in various cell types using TOOLSFect

TOOLSFect transfection reagent has higher transfection efficiencies when tested in a variety of tumor cell lines. The transfection is performed in 12-well plate format (1×10^5 cells/well) using 3 µg plasmid/well following the protocol. The percentage of GFP-positive cells was determined by FACS analysis 48 hours post transfection.

Morphology	Cell line	Transfection efficiency %	Organism / Cell type
Epithelial	H4	97.69	Homo sapiens / Neuroglioma
Glioblastoma	U87MG	82.56	Homo sapiens / Glioblastoma
Glioblastoma	U118MG	80.24	Homo sapiens / Glioblastoma
Glioblastoma	GL-261	62.59	Mus musculus / Glioblastoma
Fibroblast	9L/lacZ	78.29	Rattus norvegicus / Gliosarcoma
Glial	RG2	55.57	Rattus norvegicus / Glioma
Fibroblast	C6	86.98	Rattus norvegicus / Glioma
Fibroblast	T98G	97.88	Homo sapiens / Glioblastoma
Epithelial	CHO-K1	68.10	Cricetulus griseus / Ovary
Epithelial	HT1080	97	Homo sapiens / Fibrosarcoma
Fibroblast	COS-7	80.47	Cercopithecus aethiops / Kidney
Epithelial	MBR	76.41	Mus musculus / Epithelial