

MegaTran 2.0 - An Efficient and Economical DNA Transfection Reagent Application Guide

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Package Content and Storage Conditions

SKU	Components	Storage Condition	Shipping Condition
TT210002	1 vial of MegaTran 2.0, 0.5 mL	+4°C	Room temperature
TT210003	1 vial of MegaTran 2.0, 1 mL	+4°C	Room temperature
TT210003P5	5 vials of MegaTran 2.0, 1 mL	+4°C	Room temperature

NOTE: FOR RESEARCH PURPOSES ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USAGE.

Related Products

[Expression cDNA clones/vectors](#) - Tagged and untagged, ready for transfection

[shRNA plasmids](#)- Human, Mouse and Rat

[CRISPR vectors, gene knockout kits](#)

For technical assistance, contact OriGene at 1-888-267-4436 (301-340-3188 outside the US) or write to us at techsupport@origene.com

Introduction

MegaTran 2.0 is an efficient and versatile reagent for gene delivery that can be used for in vitro transfections. It is a non-lipid polymer-based reagent, making it an excellent choice of transfection reagent for broad cell spectrum. MegaTran 2.0 effectively condenses DNA for highly efficient gene-delivery via endosomal uptake and protects the transfected DNA from lysosomal degradation.

The major advantages of MegaTran 2.0 include:

- **High efficiency:** Ideal for transient or stable transfection in cell lines.
- **Simple application:** Perform better with serum-containing media; no requirement for media changes at time of transfection.
- **Very affordability:** economical choice for plasmid DNA transfection.

Experimental Procedures

Important Guidelines for Transfection using MegaTran 2.0:

1. MegaTran 2.0 was formulated for DNA transfection ONLY!
2. For high efficiency and lower toxicity, transfect cells at high density. 70~80% confluency is highly recommended at the time of transfection.
3. To lower cytotoxicity, transfect cells in the presence of serum (10%) and antibiotics

A General Protocol for Transfecting Adherent Cells

A sample protocol is listed here for experiments performed in 24-well plates. If performing experiments in other cell culture plates, simply multiply the suggested quantities by the relative surface area of your plate.

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 80% confluency at the time of transfection.

Note: High serum levels (>5%) with antibiotics usually do not have inhibitory effect on transfection efficiency.

Step II. Transfection

For most cell types, the optimal ratio of MegaTran 2.0 : DNA is around 3:1 (uL:ug). We recommend the MegaTran 2.0 (uL):DNA (ug) ratio of 3:1 as a starting point which usually gives satisfactory transfection efficiency with invisible cytotoxicity. To ensure the optimal size of complex particles, we recommend using serum-free DMEM to dilute DNA and MegaTran 2.0 Reagent. **Note: Don't use Opti-MEM!**

The following protocol is for transfection in 24-well plates, refer to **Table 1** for transfection in other culture formats.

1. Change fresh media: Replace with 0.5 mL of complete medium containing serum and antibiotics 30~60 minutes before transfection.
2. For each well, dilute 0.5 µg of DNA into 50 µl of serum-free DMEM. Gently pipette up and down or vortex briefly to mix.
3. Add 1.5 µl of MegaTran 2.0 reagent into the diluted DNA (not the reverse order). Gently pipette up and down or vortex briefly to mix. Incubate for 10~15 minutes at room temperature to allow transfection complexes to form.

Note: Never use Opti-MEM to dilute MegaTran 2.0 reagent and DNA, it contains serum and will disrupt transfection complex formation.

Note: Never keep the MegaTran 2.0/DNA complexes longer than 20 minutes at room temperature.

4. Add the mixture prepared in step 3 drop-wise onto cells. Gently rock the plate back-and-forth and from side-to-side to distribute the complex evenly. Incubate cells at 37°C.
5. 12~18 hours post transfection, remove transfection complex-containing media and

replace with fresh complete serum/antibiotics containing media. **For sensitive cells, to lower the cytotoxicity, change the media 5 hours after adding the transfection complex.**

6. Check transfection efficiency 24 to 48 hours post transfection.

Culture Dish	Culture Media (mL)	Plasmid DNA (ug)	Diluent Volume (uL)	MegaTran 2.0 Volume (uL)
48-well	0.3	0.25	50	0.75
12-well	0.75	0.75	76	2.25
6-well	1.0	1	100	3.0
35 mm dish	1.0	1	100	3.0
60 mm dish	2.8	2.5	200	7.5
10 cm dish	5.0	5	500	15

Table 1. Recommended Amounts for Different Culture Plates

A Protocol for Transfecting Suspension Cells

Follow the procedure below to transfect suspension 293 or CHO cells in a 30 ml volume. If you wish to transfect the suspension cells in a larger volume, scale up the transfection conditions in proportion to the culture volume.

Step I. Preparation

The day before transfection, determine the numbers of the cells and grow suspension 293 or CHO cells so that at the day of transfection (roughly 24 hours after) the cell density reaches 3×10^7 cells in total 30 mL standard culture medium.

Step II. Transfection

1. At the day of transfection, count cell viability and adjust cell density at 1.0×10^6 per mL in total 30 mL (total 3×10^7 cells) standard culture medium. Place the shaker flask containing cells in a 37°C incubator on an orbital shaker.

Important: For best results, make sure to have a single-cell suspension. It may be necessary to vortex the cells vigorously for 10–30 seconds to break down cell clumps. The viability of cells must be $>90\%$.

2. For each transfection, prepare lipid-DNA complexes as follows:
 - a. Dilute 25 μg of plasmid DNA in serum free DMEM to a total volume of 1 mL. Vortex to mix
 - b. Dilute 60 μL of MegaTran 2.0 reagent in serum free DMEM to a total volume of 1 mL. Vortex to mix.

Note: Never use Opti-MEM to dilute plasmid and MegaTran 2.0 because trace of serum from Opti-MEM may interfere formation of lipid-DNA complex.

- c. Add diluted MegaTran 2.0 reagent to the diluted DNA right away at all once to obtain total volume of 2 mL transfection mix. Vortex to mix.
- d. Incubate for 10 minutes at room temperature to allow the formation of DNA-MegaTran 2.0 complexes.

Important: Never leave the DNA-MegaTran 2.0 complex longer than 20 minutes at RT before addition to suspension 293 or CHO cells.

- 3. Add the 2 mL of DNA-MegaTran 2.0 complex to each shaker flask containing 30-mL suspension 293 or CHO cells.
- 4. Incubate the cells in a 37°C incubator with a humidified atmosphere of 8% CO₂ in air on an orbital shaker rotating at 125 rpm.
- 5. Harvest cells or media (if recombinant protein is secreted) at around 48 hours post-transfection and assay for recombinant protein expression.