

User's Guide

MegaTran 1.0 Transfection Reagent

Package Contents and Storage Conditions	2
Related products.....	2
Introduction.....	2
Production and Quality Assurance:	3
Experimental Procedures.....	3
Transfection Optimization	3
Application Protocols	4
Protocol for transient transfection	4
Protocol for stable transfection:.....	4
Troubleshooting Guide	4
Low transfection efficiency	4
High cellular toxicity	5
Frequently Asked Questions.....	5

Package Contents and Storage Conditions

The package contains one of the following components:

TT200002- MegaTran 1.0 (0.5ml)
TT200003- MegaTran 1.0 (2ml)
TT200004- MegaTran 1.0 (5X2ml)
TT200005- MegaTran 1.0 (100ml)

The above component is shipped with blue ice, but should be kept at -20°C for long-term storage. If properly stored, this product will have a 12-month shelf life.

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

Related products

- TrueClones
Expression-ready full-length cDNA clones covering 98% of human genome. <http://www.origene.com/cDNA>
- HuSH-29
Potent shRNA constructs for expression knockdown, covering 100% of human and mouse genomes. <http://www.origene.com/rna/>
- GFC-Transfection Arrays
Hundreds or thousands of unique cDNA clones arrayed individually in multi-well plates for high-throughput functional screening. <http://www.origene.com/cdna/gfc-array/>
- TurboFectin 8.0 Transfection Reagent
A new generation of transfection reagent optimized for nucleic acid delivery into eukaryotic cells in both forward and reverse transfections. <http://www.origene.com/cdna/turbofectin.msp>

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 1.0 is an efficient and versatile agent for gene delivery that can be used for *in vitro* transfections. It is a non-lipid polymer-based reagent with relatively low toxicity on transfected cells, making it an excellent choice of transfection reagent. MegaTran 1.0 effectively condenses DNA for highly efficient gene-delivery via endosomal uptake and protects the transfected DNA from lysosomal degradation.

The major advantages of MegaTran 1.0 include:

- High efficiency: Ideal for transient or stable transfection in cell lines.

- Low toxicity: The transfected cells remain healthy and produce more transgene protein.
- Simple application: Suitable for serum-containing media; no requirement for media changes.
- Extreme affordability: Great cost-saving at the price of \$1520 for 10ml.

Production and Quality Assurance:

MegaTran 1.0 is lot tested against HEK293 cell line to assure consistency and efficacy. Semiconfluent HEK293 cells in 96-well plate are transfected with 100ng of a TGFP-expression plasmid using 0.3ul of MegaTran. Over 90% of the cells expressed the green fluorescent protein 48 hrs post transfection.

MegaTran 1.0 reagent is manufactured and packaged in sterile condition and guaranteed to be free of microbial contamination.

Experimental Procedures

Transfection Optimization

Although the transfection protocol below has been shown to result in highly efficient transfection, it is encouraged to carefully optimize the reaction conditions for each individual cell type. The following variables should be considered:

- A. Cell density (% confluence at transfection): The recommended confluence for most adherent cell types at transfection is 50-70%. We recommend that customers determine the optimal cell density for the particular cell type used in their experiments.
- B. DNA purity and concentration: OriGene recommends highly purified, sterile DNA prepared on ion-exchange columns. It is important to remove the contamination of endotoxin from the DNA prep for maximal transfection efficiency. The optimal DNA concentration for transfection is 1 ug per well for a 24-well plate of HEK293 cells. Refer to Table 1 for more details.
- C. MegaTran 1.0 to DNA ratio: The standard ratio is 3 uL of MegaTran 1.0 to 1 ug of DNA. We recommend that you optimize this ratio by adjusting the volume of MegaTran 1.0 from 2-8 uL per 1 ug DNA.

Table 1. Recommended starting transfection conditions for MegaTran 1.0

<u>Tissue Culture Vessel</u>	<u>Growth area, cm²/well</u>	<u>ug of DNA</u>	<u>Ratio of MegaTran:DNA</u>
96-well plate	0.3	0.05-0.25	3:1
24-well plate	2	0.25-1.25	3:1
12-well plate	4	0.5-2.5	3:1
6-well plate	9.5	1-5	3:1
35 mm plate	8	1-5	3:1
60 mm plate	20	2-10	3:1
100 mm plate	60	5-25	3:1

Application Protocols

Protocol for transient transfection

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. See Table 1 for more details.

1. Preparation of cells

Plate $\sim 5 \times 10^4$ adherent cells or $\sim 5 \times 10^5$ suspension cells per well 24 hours prior to transfection.

2. Preparation of the MegaTran 1.0/DNA Complexes:

(Prepare immediately prior to transfection)

Dilute 1 μ g of DNA in 100 μ L of Opti-MEM I (Gibco 51985). Vortex gently.

Add 3 μ L of MegaTran 1.0 to the diluted DNA (not the reverse order) and vortex the solution immediately for 10 seconds.

Incubate for 10 minutes at room temperature.

Note: We recommend starting with the ratios of MegaTran 1.0 and DNA listed in Table I; however, subsequent optimization may further increase the transfection efficiency.

3. Transfection

Add the MegaTran 1.0/DNA mixture from step 2 to each well (already containing about 900 μ L culture medium) gently.

Generally, the volume of the MegaTran 1.0/ DNA mixture represents 1/10 of the total volume of the culture medium.

Gently rock the plate to achieve even distribution of the complexes. Incubate at 37°C for 24-48 hours.

Note: The above incubation is designed for transfection without a media change. If a media change is preferred, incubate for 30 minutes (if centrifugation is possible) or for 3- 4 hours (if centrifugation is not possible). Replace the media with the fresh complete growth media. Incubate for 24-48 hours.

Expression of the transgene can often be detected in as little as 24-48 hours post-transfection.

Protocol for stable transfection:

Perform a transfection as described above (protocol for transient transfection). Twenty-four hours post-transfection, passage the cells (at 1:10 or higher dilution) into fresh growth medium containing selective medium. A mock transfection should be performed in parallel as a control. Grow and passage the cells as necessary, maintaining selection pressure by keeping the selective agent in the growth medium. After 1-2 weeks, a large number of the cells will be killed; the cells that remain growing in the selective medium have retained the expression plasmid, which stably integrates into the genome of the targeted cells.

Troubleshooting Guide

Low transfection efficiency

Possible cause: Inefficient complex formation.

Recommended solution: Always vortex the mixture immediately after the addition of the reagent to DNA.

Possible cause: Suboptimal reagent/DNA ratio.

Recommended solution: Optimize the quantity of transfection reagent added to the fixed amount of DNA.

Possible cause: Suboptimal quantity of DNA.

Recommended solution: Optimize the amount of DNA used for transfection. Keep the transfection reagent/DNA ratio constant

Possible cause: Poor polyplex/cell surface contact.

Recommended solution: Gently centrifuge the culture plates.

Possible cause: Poor DNA quality.

Recommended solution: Use high quality DNA with an A260/A280 ratio greater than 1.8.

Possible cause: Suboptimal cell confluency.

Recommended solution: Optimize cell plating conditions. Ensure that adhered cells are 50-70% confluent at the time of transfection. Ensure that suspension cells are in logarithmic growth phase at the time of transfection.

Possible cause: Mycoplasma contamination.

Recommended solution: Mycoplasma infection in cell culture often results in poor and/or non-reproducible transfection. Regularly check your cells for mycoplasma infection.

High cellular toxicity

Possible cause: Toxic transgene.

Recommended solution: Verify if the expressed transgene is toxic.

Possible cause: Suboptimal incubation conditions.

Recommended solution: Reduce incubation time of the polyplexes with the cells. Replace the transfection mixture 3-6 hours later with fresh growth medium.

Possible cause: Suboptimal quantity of DNA.

Recommended solution: Reduce the quantity of DNA used for transfection.

Possible cause: Cell density is too low.

Recommended solution: Increase the plating density of cells used for transfection.

Frequently Asked Questions

Q: What kind of reagent is MegaTran 1.0?

A: **MegaTran 1.0** is a brand new non-lipid polymer based transfection reagent specially designed and manufactured for large volume DNA transfections.

Q: What cell lines have been tested for use with this reagent?

A: We have successfully tested CHO; 293; Sf9; HeLa COS-7; and NIH3T3 cells for high efficiency transfection with MegaTran 1.0.

Q: Has this reagent been optimized for any cell type in particular?

A: MegaTran 1.0 has been optimized for the HEK293 and CHO cell lines as these are commonly used for high levels of protein production.

Q: Should I use the same DNA: MegaTran ratio for cell types that have not been tested?

A: We suggest that you use our optimal ratio as a starting point and test several bracketed ratios of DNA to MegaTran 1.0.

Q: What level of efficiency should I expect?

A: When HEK293 and CHO cells are used, over 90% of the cells can be transfected. However, with different cell lines and different DNA preparation, the efficiency varies. Each of them needs to be optimized individually.

Q: What level of toxicity should I expect?

A: MegaTran 1.0 display much less toxicity compared to the commonly used Lipofectamine. With the recommended protocol using CHO cells, the transfected cells appear to be healthy morphologically and continue to produce the transgene protein even at day 6 post transfection.

Q: Is the transfection efficiency sensitive to cell density?

A: Yes. The best transfection efficiency is usually achieved with 60-80% confluent cells. Different cell lines need individualized optimization for the best condition.

Q: Can MegaTran1.0 be used to transfect primary cells?

A: OriGene has not tested MegaTran on any primary cells.

Q: Can MegaTran1.0 be used in serum-free media?

A: MegaTran 1.0 works efficiently in media with or without serum.

Q: Will antibiotics in the growth medium interfere with transfection?

A: MegaTran 1.0 works well in media containing antibiotic and anti-mycobacterial agents.

Q: Do I need to replace the media after transfection?

A: No. One benefit of MegaTran is that there is no need to change the media following transfection.

Q: What are the storage conditions and stability of this reagent?

A: We recommend the storage condition as 4⁰C.

Q: Do I need to add a separate solution to pre-complex DNA like I need to do for other reagents?

A: No. Unlike some competitive reagents, there is no need to pre-complex DNA as the DNA associates quite well with MegaTran 1.0.

Q: What is the recommended method for the quantitation of my DNA?

A: We recommend using UV spectrophotometers to obtain the accurate quantity and the purity of the DNA samples.

Q: Where can I read more about this reagent?

A: Please access the product documentation and data on our website at the following address: www.origene.com/cdna/megatran1.msp.

Q: What is the composition of this reagent? Does it contain EDTA or any other chelating agents? Does it contain any protein?

A: No. There are no chelating agents or protein in MegaTran 1.0.