

Altogen Biosystems

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293 Transfection Protocol (Kit for Embryonic Kidney Cells, CRL-1573)

Catalog No. 1151 Size: 0.5 ml
Catalog No. 1152 Size: 1.5 ml
Catalog No. 7014 Size: 1.5 ml

Contents and Shipping:

293 Transfection Kit includes 293 Transfection Reagent (0.5 ml / 1.5 ml / 8.0 ml), Transfection Enhancer (0.5 ml), and Complex Condenser (0.5 ml). 293 Transfection Reagent is supplied in liquid form at a concentration of 0.3 mg/ml, shipped at ambient temperature.

Description:

Two component lipid-based proprietary formulation optimized for 293 cells transfection.

Product Qualification:

293 Transfection Reagent is tested functionally by transfection of 293 cells with a small interfering RNAs targeting 3 different genes (Lamin A/C, GAPDH, Cyclophilin B). Transfection Reagent is tested for absence of nuclease contamination and microbial contamination.

Storage:

Store reagent at 4°C upon receipt. If stored properly, reagent is stable for 12 months.

Intended Use:

For *in vitro* use only.

Safety Data Sheet (SDS):

SDS documents are available online at <https://altogen.com>

In Vivo Transfection Kits (for compound testing in rodents):

- Catalog #5010 / 5011 / 5012 - Lipid *In Vivo* Transfection Kit
- Catalog #5020 / 5021 / 5022 - Polymer *In Vivo* Transfection Kit
- Catalog #5030 / 5031 / 5032 - Nanoparticle *In Vivo* Transfection Kit
- Catalog #5040 / 5041 / 5042 - PEG-Liposome *In Vivo* Transfection Kit
- Catalog #5050 / 5051 / 5052 - Pancreas *In Vivo* Transfection Kit
- Catalog #5060 / 5061 / 5062 - Liver *In Vivo* Transfection Kit
- Catalog #5070 / 5071 / 5072 - Kidney *In Vivo* Transfection Kit

Transfection Controls and Recommended Products:

- Catalog #4060 - GFP-expressing plasmid DNA (25 ug)
- Catalog #4061 - Cell Cycle Arrest siRNA (5 nmol)
- Catalog #4062 - Apoptosis Inducing siRNA (5 nmol)

To Place an Order:

Both domestic (USA) and international orders can be placed online (<https://altogen.com>) using credit card payment. Purchase Order (PO) can be faxed at (702) 989-0841 (for USA only).

Transfection Resource: altogen.com/transfection-resource

Altogen Labs. GLP Compliant Pre-clinical CRO Laboratory Services:

AltogenLabs.com

Recommended Transfection Protocols (for 24-well plate):

| 293 Standard Transfection Protocol (24-well plate): | 293 Reverse Transfection Protocol (24-well plate): |
|---|--|
| <ol style="list-style-type: none">1. Plate 10,000 - 15,000 293 cells per well in 0.5 ml of complete growth medium 12–24 hours prior to transfection2. Wash with 1xPBS and add 0.5 ml of fresh growth medium3. Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and<ul style="list-style-type: none">• 750 ng DNA (or mRNA), or• 30 nM - 50 nM of siRNA (or microRNA)<i>*Referred to a final volume including growth medium</i>4. Incubate transfection complexes at RT for 15 - 30 minutes5. <u>Optional</u>: Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity6. Add prepared transfection complexes to 0.5 ml of complete growth medium with 293 cells (from step 2)7. Incubate cells at 37°C in a humidified CO₂ incubator8. Assay for phenotype or target gene expression 48 - 72 hours after transfection | <ol style="list-style-type: none">1. Prepare 293 cell suspension:<ol style="list-style-type: none">a. Trypsinize cells (0.05% Trypsin) for 3-5 minutes at 37°Cb. Dilute in complete growth medium to 5 x 10⁴ cells/ml2. Prepare transfection complexes by mixing 40 µl of serum-free medium, 5.5 µl of transfection reagent, and<ul style="list-style-type: none">• 750 ng DNA (or mRNA), or• 30 nM - 50 nM of siRNA (or microRNA)<i>*Referred to a final volume including growth medium</i>3. Incubate transfection complexes at RT for 15 - 30 minutes4. <u>Optional</u>: Add 2 µl of Complex Condenser. This reagent reduces the size of transfection complex, therefore increasing transfection efficiency; however it may increase cell toxicity5. Plate 20,000 - 30,000 cells per well in 0.5 ml of complete growth medium (from step #1) into culture plate6. Add prepared transfection complexes (from step 3 or 4)7. Incubate cells at 37°C in a humidified CO₂ incubator8. Assay for phenotype or target gene expression 48 - 72 hours after transfection |
| <u>Optional</u> : Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection | <u>Optional</u> : Transfection efficiency can be increased by addition of Transfection Enhancer reagent. Add 2 µl of Transfection Enhancer reagent 12-24 hours after transfection |
| If the viability of 293 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant | If the viability of 293 cells being transfected is affected at 16 - 24 hours post-transfection, the level of cytotoxicity can be decreased by changing the growth medium and eliminating redundant exposure of cells to transfectant |

Scaling Up or Down Transfections:

| Culture Vessel Surface Area (cm ²) | Volume of Growth Medium (ml) | Transfection Reagent (µl) | Complex Condenser (µl) | Transfection Enhancer (µl) |
|--|------------------------------|---------------------------|------------------------|----------------------------|
| 96-well, 0.3 cm x cm | 0.12 | 1.5 | 0.3 | 0.3 |
| 24-well, 2 cm x cm | 0.5 | 5.5 | 2 | 2 |
| 12-well, 4 cm x cm | 1 | 12 | 4 | 4 |
| 6-well, 10 cm x cm | 3 | 35 | 12 | 12 |
| 60-mm, 20 cm x cm | 5 | 60 | 20 | 20 |
| 10-cm, 60 cm x cm | 15 | 180 | 60 | 60 |

Optimizing Transfection:

To obtain the highest transfection efficiency, optimize transfection conditions by varying 293 cell density and amount of transfection reagent. High passage of 293 cells and use of antibiotics (or growth factors) may require using larger volumes of 293 transfection reagent per reaction.

Limited Use Label License:

The purchase of this product conveys to the purchaser the limited right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. This product is for research purposes only and is not for use in commercial applications of any kind. For information on obtaining additional rights, please contact Altogen Biosystems at orders@altogen.com.

Limited Product Warranty:

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Altogen Biosystems. Altogen Biosystems shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product. This product is developed, manufactured and sold for research purposes only. Reagent is not suitable for administration to humans.