

# TrojanPORTER™



## Transfection Reagent-SAMPLE ONLY

Catalog #	Content	Amount
T901001 (25 reactions)	TrojanPORTER™ Transfection Reagent	50 µl

<b>Shipping</b>	Shipped at room temperature.
<b>Storage</b>	Store at 4°C.

RELATED PRODUCTS (Continued)	Catalog Numbers
TrojanPORTER™ Transfection Reagent	T901007, 375 reactions (0.75 ml)
	T901015, 750 reactions (1.5 ml)
	T901075, 3750 reactions (5 x 1.5 ml)
	T901150, 7500 reactions (10 x 1.5 ml)
Detachin™ Cell Detachment Solution	T100100, 100 ml
	T100110, 10 x 100 ml
Expresso™ Mammalian Cells, HeLa	C501100, Single-shot (1 x 10 <sup>7</sup> cells)
	C501200, Double-shot (2 x 10 <sup>7</sup> cells)
Expresso™ Mammalian Cells, CHO-K1	C502100, Single-shot (1 x 10 <sup>7</sup> cells)
	C502200, Double-shot (2 x 10 <sup>7</sup> cells)
Expresso™ Mammalian Cells, HEK 293	C503100, Single-shot (1 x 10 <sup>7</sup> cells)
	C503200, Double-shot (2 x 10 <sup>7</sup> cells)
Expresso™ Mammalian Cells, NIH-3T3	C504100, Single-shot (1 x 10 <sup>7</sup> cells)
	C504200, Double-shot (2 x 10 <sup>7</sup> cells)
Expresso™ Mammalian Cells, COS-7	C505100, Single-shot (1 x 10 <sup>7</sup> cells)
	C505200, Double-shot (2 x 10 <sup>7</sup> cells)

**Introduction:** TrojanPORTER™ is a convenient biodegradable cationic polymer that allows for simple same day, high efficiency, and low cost gene delivery in mammalian cell lines. During transfection, the polymer/DNA complexes (polyplexes) are endocytosed into the cells, where the polymer is biodegraded into small non-toxic molecules. The ability of TrojanPORTER to biodegrade *in vivo* dramatically reduces its cytotoxicity while maximizing the delivery of macromolecules into cells. The protocol is easy! Simply plate your cell line in complete medium, and immediately add the DNA/TrojanPORTER polyplexes without the need for overnight incubation. Assay for transgene expression 24-48 hours later. TrojanPORTER™ is compatible with serum-containing medium, it is simple to use, and it provides high transfection efficiency at a low cost per reaction relative to other commercial transfection reagents.

## Methods and Procedures

### A. Preliminary Notes

- TrojanPORTER Transfection Reagent is provided at a stock concentration of 5X. We recommend using it at the stock concentration; but it can be diluted to 1X for easier pipetting and transfection optimization. Use only sterile water or serum-free medium (SFM) for dilution. Also, use TrojanPORTER within 30 minutes if diluted in SFM.
- The following transfection protocol is optimized with the Achilles GFP Control Plasmid and Detachin™ cell Detachment Solution (available separately from Genlantis for the Sample kit). Note that both Achilles GFP Plasmid and Detachin solution are included in all other TrojanPORTER Kits.
- We recommend that users optimize DNA amount used, DNA:polymer ratios, cell plating densities, and timing of transfection for different types of cell lines and culturing conditions used.

### B. Preparation of Adherent Mammalian Cells for Transfection

- Passaged cells should be maintained in culture in log phase growth. Cells should be harvested at no greater than 80% confluency for optimal transfection results.
- Depending on your application, frozen cells may be thawed and cultured in complete medium overnight then plated and transfected the next day with TrojanPORTER.  
**Note:** We recommend that cultured cells be maintained in *antibiotic-free* medium to maximize transfection efficiency.
- Remove medium from cells by aspiration.
- Wash away residual medium with 1X PBS then aspirate.
- Add room temperature trypsin or Detachin Cell Detachment Solution at 1.0 ml per 75cm<sup>2</sup> of surface area. Cover entire surface by gently rocking culture vessel. Incubate at 37°C in 5% CO<sub>2</sub> for a maximum of 5 minutes (times will vary depending on cell line).
- Confirm all cells are detached under a light microscope.

- To inactivate Trypsin or Detachin™, use a volume of complete plating medium equal to 5 X the volume of cell detachment solution used. Pipette up and down 3 times then transfer cells to appropriate sterile conical tube.
- Centrifuge for 5 minutes at 2,000g.
- Aspirate medium off carefully without disturbing the pellet.
- Resuspend cell pellet into a single cell suspension using complete medium. Typically, a 75-85% confluent T150 cell pellet is resuspended in 1 ml of medium for counting.
- Determine the cell count/ml.
- Plate adherent cells in complete medium at the recommended densities in **Table 1** below:

**Table 1: Recommended Adherent Cells Densities**

Culture Vessel	Recommended Plating Density and Volume	
	Cells/ml	µl/well
96-well	2.0–4.0 x 10 <sup>5</sup>	100 µl
24-well	1.5–2.5 x 10 <sup>6</sup>	500 µl
12-well	2.0–5.0 x 10 <sup>6</sup>	1,000 µl
6-well	8.0–10.0 x 10 <sup>6</sup>	2,000 µl

- Incubate plated cells at 37°C in 5% CO<sub>2</sub>, while you prepare the transfection reaction.

### C. Preparation of Suspension Mammalian Cells for Transfection

**Notes:** For optimal transfection results, passaged suspension cells should be maintained and harvested in log phase growth. We recommend that suspension cells be maintained in antibiotic-free medium to maximize transfection efficiency.

- Harvest suspension cells; centrifuge for 5 min. at 2,000g.
- Aspirate off medium being careful not to disturb the pellet.
- Resuspend pellet into a single cell suspension with complete medium, then determine cell count

- Plate suspension cells in complete medium at the recommended densities in **Table 3** below.

**Table 3: Recommended Suspension Cells Plating Conditions**

Culture Vessel	Recommended Densities and Volumes	
	Cells/ml	µl/well
96-well	1.0–2.0 x 10 <sup>6</sup>	100 µl
24-well	7.5–12.5 x 10 <sup>6</sup>	500 µl
12-well	1.0–2.5.0 x 10 <sup>7</sup>	1,000 µl
6-well	4.0–5.0 x 10 <sup>7</sup>	2,000 µl

- Incubate plated cells at 37°C in 5% CO<sub>2</sub>, while you prepare the transfection reaction.

### D. Transfection Procedure

- Prepare DNA and TrojanPORTER at a 1:2 ratio (W:V) in serum-free medium (SFM) in separate tubes using the following procedure and the amounts in **Table 4** below:
  - Combine DNA and serum-free medium and incubate for 5 minutes @ room temperature.
  - Combine TrojanPORTER and serum-free medium and incubate for 5 minutes @ room temperature.
  - Combine 1a and 1b and incubate for 20 minutes at room temperature to form polyplexes. Do not exceed 30 minutes, as transfection efficiency will decline.
- Add polyplexes directly to plated cells from step B 13 or C 5.
- Incubate @ 37°C in 5% CO<sub>2</sub> for 24-48 hours before performing downstream analysis of transgene expression.

**Table 4: Recommended Transfection Conditions at 1:2 (w:v) ratios**

Culture Vessel	DNA Amount and Volume		TrojanPORTER Volume (at 5 µg/µl)	
	DNA (µg)	Volume in SFM (µl)	TrojanPORTER (µl)	Volume in SFM (µl)
96-well	0.5	50	1.0	50
24-well	1.0	250	2.0	250
12-well	1.5	500	3.0	500
6-well	4.0	1000	8.0	1000

### TROUBLESHOOTING

Problem	Causes	Possible Solutions
Low Transfection Efficiency	Cell Density	If using freshly detached and plated cells, use higher plating density. Optimal cell seeding density should be determined per cell line.
	DNA Concentration	Try increasing or decreasing the amount of DNA used by 50% for each reaction size. For example, use 0.5 and 1.5 µg of DNA per transfection in a 24 well plate per 2.0 µl of TrojanPORTER.
	DNA to TrojanPORTER Ratio	Try optimizing DNA:TrojanPORTER by trying 1:2, 1:3, or 1:4 ratios (W:V).
	Incubation Time	Try longer incubation times, such as 48 hours after transfection, to improve results.
High Cytotoxicity	Cell Condition	Use only healthy log phase cells passaged at least 24 hours before transfection. Also use Detachin to maximize cell health and maintain without antibiotics before transfection. If you are using Trypsin, try the Detachin™ Cell Detachment Solution (Cat #: T100100)
	Cell Density	TrojanPORTER requires relatively higher cell density for transfection.
	DNA Concentration	Avoid excessive DNA amounts that may cause cell death; do not exceed 1:4 ratio of DNA:TrojanPORTER (w:v).
Poor Cell Condition	Mycoplasma Contamination	Test cultured cell lines for mycoplasma contamination. We recommend the MycoScope™ Kit (MY01050).
	Cross-contamination	Work with only one cell line at a time to maintain optimal cell health and achieve optimal transfection efficiency and expression. Start with fresh stock of cells if cross-contamination is a possibility.

**LIMITED LICENSE:** The purchase price paid for the TrojanPORTER™ Transfection Reagent Kit (hereto "TrojanPORTER") grants end users a non-transferable, non-exclusive license to use the kit and/or its components **for internal research use only** as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS) -- separate licenses are available for non-research use or applications. TrojanPORTER and/or its components are not to be used for human diagnostic or included/used in any drug intended for human use. Care and attention should be exercised in handling the kit components by following appropriate research laboratory practices and kit instructions. Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using this kit, you agree to be bound by the terms of this license as governed and enforced by the laws of the State of California.