

PORT-folio™ 96



A division of Gene Therapy Systems, Inc.

Transfection Optimization Plate

Catalog #	Content	Amount
TPF2096	PORT-folio 96 Transfection Optimization Plate.	1 plate (reagents are provided in solution)
	Diluent	500 µl

Shipping	Shipped at room temperature.
Storage	Store at 4°C; stable for 6 months at 4°C.

INTRODUCTION The PORT-folio™ 96 Transfection Optimization Plate enables you to quickly and easily determine the optimal transfection and expression conditions for your specific delivery molecule (DNA, siRNA, Oligo, etc.) of choice. Genlantis has done all of the upfront work for you by providing our best transfection reagents titrated in the optimal recommended range in a tissue culture-treated 96-well plate. This process uses a rapid reverse transfection protocol with 3 easy steps: 1) prepare your delivery molecule; 2) add directly to the PORT-folio 96 Transfection Optimization Plate and incubate; and 3) add freshly detached cells.

Once you determine the optimal transfection reagent, reagent amount, and delivery molecule amount, contact Genlantis Customer or Technical Services and order your reagent of choice. Genlantis, the molecular delivery experts, now makes your transfection optimization as easy as 1-2-3.

RELATED PRODUCTS	Catalog Numbers
GenePORTER®-H Transfection Reagent	T201007H, 0.75 ml (75 reactions)
	T201015H, 1.5 ml (150 reactions)
	T201075H, 5 x 1.5 ml (750 reactions)
GenePORTER® 2 Transfection Reagent	T202007, 0.75 ml (75 reactions)
	T202015, 1.5 ml (150 reactions)
	T202075, 5 x 1.5 ml (750 reactions)
TrojanPORTER™ Transfection Reagent	T901007, 0.75 ml (375 reactions)
	T901015, 1.5 ml (750 reactions)
	T901075, 5 x 1.5 ml (3,750 reactions)
GeneSilencer® siRNA Transfection Reagent	T500750, 0.75 ml (200 reactions)
	T505750, 5 x 0.75 ml (1,000 reactions)
NeuroPORTER® Transfection Reagent	T400150, 1.5 ml (75-300 reactions)
	T400750, 5 x 1.5 ml (375-1500 reactions)
BaculoPORTER™ Transfection Reagent	T701007, 0.75 ml (150 reactions)
	T701035, 5 x 0.75 ml (750 reactions)
BioPORTER® Protein Delivery Reagent	BP502424, 24 single use tubes.
	BP509696, 96 single use tubes.
NeuroFECT™ Transfection Reagent	T800075, 0.75 ml (75-300 reactions.)
	T800750, 5 x 0.75 ml (375-1500 rxns.)
Cytfectin™ Transfection Reagent (Oligos)	T610001 (1 ml)
	T610005 (5 x 1 ml)
PrimaPure™ Primary Cells	Please call for details or visit our website
NeuroPURE™ Neuronal Cells	Fresh live neuronal cells. Please call for details or visit our website
Detachin™ Cell Detachment Reagent	T100100 (100 ml)
	T100110 (10 x 100 ml)

PORT-Folio 96 Well Plate Layout

Reagent	DNA, µg	1.0	0.5	0.25	1.0	0.5	0.25	1.0	0.5	0.25	1.0	0.5	0.25
Well		1	2	3	4	5	6	7	8	9	10	11	12
GP-H	A	3	3	3	2	2	2	1	1	1	0.5	0.5	0.5
GP2	B	3	3	3	2	2	2	1	1	1	0.5	0.5	0.5
GP2 D	C	3	3	3	2	2	2	1	1	1	0.5	0.5	0.5
GS	D	3	3	3	2	2	2	1	1	1	0.5	0.5	0.5
GS D	E	3	3	3	2	2	2	1	1	1	0.5	0.5	0.5
TP	F	4	4	4	3	3	3	3	3	3	2	2	2
NP	G	4	4	4	3	3	3	3	3	3	2	2	2
BacP	H	4	4	4	3	3	3	3	3	3	2	2	2

Numbers inside the table grid indicate the amount, in µl, of the transfection reagent present per well, within the total volume of 25 µl per well.

Legend

GP-H	GenePORTER®-H Transfection Reagent
GP2	GenePORTER® 2 Transfection Reagent
GP2 D	GenePORTER® 2 Transfection Reagent with Diluent
GS	GeneSilencer® Transfection Reagent
GS D	GeneSilencer® Transfection Reagent with Diluent
TP	TrojanPORTER™ Transfection Reagent
NP	NeuroPORTER® Transfection Reagent
BacP	BaculoPORTER® Transfection Reagent

MATERIALS AND METHODS

PORT-folio™ 96 Transfection Optimization Protocol

A-General Notes

- Store PORT-folio plate at 4°C and centrifuge before use to collect reagents at bottom of wells.
- For your convenience each transfection reagent (25µl/well) is pre-titrated at the recommended range.
- You will need a minimum of 80µg of plasmid DNA per 96 well plate
- For siRNA, we recommend testing 5, 25 and 50nM with and without diluent. Prepare master mixes according to Tables 1 & 2 below.
- This is a reverse transfection protocol, where freshly detached cells in complete medium are added directly to the transfection reagents (same-day) in the PORT-folio 96 plate.

B- Preparation of Cells for Transfection

1. The day before transfection, transfer healthy log phase cultured cells to antibiotic-free complete medium so that they are 70-80% confluent the day of transfection.

NOTE: It is preferred to omit antibiotics from the media 24 hours prior to and during transfection, because it may increase both transfection efficiency and overall expression levels.

2. Prepare cells before setting up transfection reactions. You will need a minimum of 1.2 x 10⁶ total cells for one PORT-folio 96 plate transfection.
3. Harvest cells using your standard protocol or procedure. We recommend using the Detachin™ Cell Detachment Reagent (Catalog Number: T100100) for adherent cell lines.
4. Determine the cell count.
5. Combine 1.2 x 10⁶ cells with complete medium for a final volume of 12 ml. Place at 37°C, 5% CO₂ until you are ready to transfect.

C-Preparation of DNA Master Mixes Without Diluent

6. Prepare three different DNA master mix dilutions (1.0, 0.5 and 0.25 µg/well), by combining DNA (µg) with serum-free medium (µl) as in Table 1 below:

Table 1: Preparation of DNA Master Mixes Without Diluent

DNA Master Mix	DNA (µg)	Serum-free Medium (µl)	Volume of DNA Master Mix/well (µl)
1.0 µg/well	30.0	Up to 750 µl final	25.0
0.5 µg/well	15.0	Up to 750 µl final	25.0
0.25 µg/well	7.5	Up to 750 µl final	25.0

7. Vortex to mix.

D- Preparation of DNA Master Mixes With Diluent

8. Prepare three different DNA master mix dilutions (1.0, 0.5 and 0.25 µg/well), by combining DNA (µg) with serum-free medium (µl) and diluent (µl) as in Table 2 below:

Table 2: Preparation of DNA Master Mixes with Diluent

DNA Master Mix	DNA (µg)	Diluent (µl)	Serum-free Medium (µl)	Volume of DNA Master Mix/well (µl)
1.0 µg/well	10.0	250.0	Up to 312.5 µl final	25.0
0.5 µg/well	5.0	125.0	Up to 312.5 µl final	25.0
0.25 µg/well	2.5	62.5	Up to 312.5 µl final	25.0

9. Vortex to mix.
10. Incubate 5 minutes @ room temperature.

E-Preparation of Transfection Reactions

11. Transfer 25 µl of DNA master mix without diluent to the indicated wells as in diagrams 1-3 below:

Diagram 1: 1.0 µg/well [Rows A,B,D,F,G,H] -- [Columns: 1,4,7,10]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A	■			■			■			■		
B	■			■			■			■		
C												
D	■			■			■			■		
E												
F	■			■			■			■		
G	■			■			■			■		
H	■			■			■			■		

Diagram 2: 0.5 µg/well [Rows A,B,D,F,G,H] -- [Columns: 2,5,8,11]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A		■			■			■			■	
B		■			■			■			■	
C												
D		■			■			■			■	
E												
F		■			■			■			■	
G		■			■			■			■	
H		■			■			■			■	

Diagram 3: 0.25 µg/well [Rows A,B,D,F,G,H] -- [Columns: 3,6,9,12]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A			■			■			■			■
B			■			■			■			■
C												
D			■			■			■			■
E												
F			■			■			■			■
G			■			■			■			■
H			■			■			■			■

PORT-folio™ 96 Optimization Plate Transfection Manual

12. Transfer 25 µl of DNA master mix **with diluent** to the appropriate wells as in Diagrams 4-6 below:

Diagram 4: 1.0 µg/well [Rows C,E] -- [Columns: 1,4,7,10]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Diagram 5: 0.5 µg/well [Rows C,E] -- [Columns: 2,5,8,11]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Diagram 6: 0.25 µg/well [Rows C,E] -- [Columns: 3,6,9,12]

Well	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

13. Set a multichannel pipette to 40 µl, pipette each well up and down 3X to mix the PORT-folio reagents and DNA. **Avoid introducing bubbles during the pipetting procedure.**

14. Incubate for about 15 minutes. **DO NOT Exceed 20 minutes.**

D-Reverse Transfection-Adding Cells To PORT-folio/DNA Mix

15. Pipett cells prepared in Section B into a sterile multichannel pipette basin.
16. Using multichannel pipette, aliquot 100 µl of cells (=10,000 cells/well) to each well of the PORT-folio 96 plate from Section E 14. We recommend incubating the transfection reactions plate for at least 48 hours at 37°C and 5% CO₂ prior to assaying for efficiency and/or expression.

E- PORT-folio 96 Well Transfection Results

17. Depending on your experimental goals, determine which reagent, reagent amount, and DNA amount yielded optimal results. Record your results in Table 3 below. You may decide to try more than one optimized condition for your actual experiment.

Table 3. Optimal Reagent and Reagent Volumes

Optimal Transfection Reagent	Optimal Reagent Volume, µl	Optimal Delivery Molecule Amount, µg

18. Contact our Technical Support Line at (888) 428-0558 (extension 3) for reagent purchase options, or visit our website at www.genlantis.com for reagent purchase options.

LIMITED LICENSE: The purchase price paid for the PORT-folio™ 96 Transfection Optimization Plate (hereto "PORT-folio") 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. PORT-folio 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.

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