



A division of Gene Therapy Systems, Inc.

**GenePORTER<sup>®</sup> 2 Transfection Reagent  
Cell Specific Protocols**

[HeLa](#)

[HepG2](#)

[K562](#)

[NIH-3T3](#)

[PC-12](#)



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## GenePORTER<sup>®</sup> 2 Transfection Protocol

<b>Cell Type:</b>	HeLa ( <a href="#">Return to Top</a> )
<b>Transfection Reagent:</b>	GenePORTER <sup>®</sup> 2 Transfection Reagent
<b>Tissue Culture Dish Size:</b>	24-well plate
<b>Expected Transfection Efficiency:</b>	~40% by GFP immunofluorescence

### Procedures

1. The day before transfection, plate the cells at  $1.5 \times 10^5$  cells per well.
2. For each well, dilute 3.5  $\mu$ l of GenePORTER<sup>®</sup> 2 reagent with 21.5  $\mu$ l of serum-free DME.
3. For each well, dilute 1  $\mu$ g of DNA at 1  $\mu$ g/ $\mu$ l with 25  $\mu$ l of DNA Diluent B.
4. Add the diluted DNA to the diluted GenePORTER<sup>®</sup> 2 reagent, mix well by pipetting several times and incubate at room temperature for 5-15 minutes.
5. Add 200  $\mu$ l of serum-free DME to the GenePORTER<sup>®</sup>2/DNA complexes bringing the total volume to 250  $\mu$ l for each well.
6. Aspirate the culture medium from the cells, and carefully add the GenePORTER<sup>®</sup>2/DNA mixture to the cells.
7. Incubate at 37°C for 4 hours.
8. Add one volume (250  $\mu$ l) of medium containing 20% FCS to each well.
9. Continue to incubate overnight under 5-10% CO<sub>2</sub> at 37°C.
10. Twenty-four hours post-transfection, feed the cells with 250  $\mu$ l of fresh growth media for each well.
11. Assay for gene expression 48 hours post-transfection.

Note: Transfection efficiencies may vary depending on the promoter, health of the cells, reporter gene, and method of assay.



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## GenePORTER<sup>®</sup> 2 Transfection Protocol

<b>Cell Type:</b>	HepG2 ( <a href="#">Return to Top</a> )
<b>Transfection Reagent:</b>	GenePORTER <sup>®</sup> 2 Transfection Reagent
<b>Tissue Culture Dish Size:</b>	24-well plate
<b>Expected Transfection Efficiency:</b>	>60% by GFP immunofluorescence

### Procedures

1. The day before transfection, plate the cells at  $8 \times 10^4$  cells per well in 200  $\mu$ l of serum-containing culture medium.
2. For each well, dilute 15  $\mu$ l of GenePORTER<sup>®</sup> 2 reagent with 60  $\mu$ l of OptiMem<sup>™</sup>.
3. For each well, dilute 3  $\mu$ g of DNA at 1  $\mu$ g/ $\mu$ l with 75  $\mu$ l of DNA Diluent.
4. Add the diluted DNA to the diluted GenePORTER<sup>®</sup> 2 reagent, mix well by pipetting several times and incubate at room temperature for 5-15 minutes.
5. Add the GenePORTER<sup>®</sup>2/DNA complexes directly to the cells growing serum-containing culture medium. The final transfection volume should now be 350  $\mu$ l.
6. Incubate overnight under 5-10% CO<sub>2</sub> at 37°C.
7. Twenty-four hours post-transfection, feed the cells with 250  $\mu$ l of fresh growth media for each well.
8. Assay for gene expression 48 hours post-transfection.

Note: Transfection efficiencies may vary depending on the promoter, health of the cells, reporter gene, and method of assay.



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## GenePORTER<sup>®</sup> 2 Transfection Protocol

<b>Cell Type:</b>	K562 ( <a href="#">Return to Top</a> )
<b>Transfection Reagent:</b>	GenePORTER <sup>®</sup> 2 Transfection Reagent
<b>Tissue Culture Dish Size:</b>	24-well plate
<b>Expected Transfection Efficiency:</b>	>50% by GFP immunofluorescence

### Procedures

1. The day before transfection, plate the cells at  $3.5 \times 10^4$  cells per well.
2. For each well, dilute 3.5  $\mu$ l of GenePORTER<sup>®</sup> 2 reagent with 21.5  $\mu$ l of OptiMem<sup>™</sup>.
3. For each well, dilute 1  $\mu$ g of DNA at 1  $\mu$ g/ $\mu$ l with 25  $\mu$ l of DNA Diluent B.
4. Add the diluted DNA to the diluted GenePORTER<sup>®</sup> 2 reagent, mix well by pipetting several times and incubate at room temperature for 5-15 minutes.
5. Add 200  $\mu$ l of OptiMem<sup>™</sup> to the GenePORTER<sup>®</sup>2/DNA complexes bringing the total volume to 250  $\mu$ l for each well.
6. Aspirate the culture medium from the cells, and carefully add the GenePORTER<sup>®</sup>2/DNA mixture to the cells.
7. Incubate at 37°C for 4 hours.
8. Add one volume (250  $\mu$ l) of medium containing 20% FCS to each well.
9. Continue to incubate overnight under 5-10% CO<sub>2</sub> at 37°C.
10. Twenty-four hours post-transfection, feed the cells with 250  $\mu$ l of fresh growth media for each well.
11. Assay for gene expression 48 hours post-transfection.

Note: Transfection efficiencies may vary depending on the promoter, health of the cells, reporter gene, and method of assay.



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## GenePORTER<sup>®</sup> 2 Transfection Protocol

<b>Cell Type:</b>	NIH-3T3 <a href="#">(Return to Top)</a>
<b>Transfection Reagent:</b>	GenePORTER <sup>®</sup> 2 Transfection Reagent
<b>Tissue Culture Dish Size:</b>	24-well plate
<b>Expected Transfection Efficiency:</b>	>50% by GFP immunofluorescence

### Procedures

1. The day before transfection, plate the cells at  $2 \times 10^4$  cells per well in 200  $\mu$ l of serum-containing culture medium.
2. For each well, dilute 10  $\mu$ l of GenePORTER<sup>®</sup> 2 reagent with 40  $\mu$ l of OptiMem<sup>™</sup>.
3. For each well, dilute 2  $\mu$ g of DNA at 1  $\mu$ g/ $\mu$ l with 50  $\mu$ l of DNA Diluent.
4. Add the diluted DNA to the diluted GenePORTER<sup>®</sup> 2 reagent, mix well by pipetting several times and incubate at room temperature for 5-15 minutes.
5. Add the GenePORTER<sup>®</sup>2/DNA complexes directly to the cells growing serum-containing culture medium. The final transfection volume should now be 250  $\mu$ l per well.
6. Incubate overnight under 5-10% CO<sub>2</sub> at 37°C.
7. Twenty-four hours post-transfection, feed the cells with 250  $\mu$ l of fresh growth media for each well.
8. Assay for gene expression 48 hours post-transfection.

Note: Transfection efficiencies may vary depending on the promoter, health of the cells, reporter gene, and method of assay.



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## GenePORTER<sup>®</sup> 2 Transfection Protocol

<b>Cell Type:</b>	PC-12 ( <a href="#">Return to Top</a> )
<b>Transfection Reagent:</b>	GenePORTER <sup>®</sup> 2 Transfection Reagent
<b>Tissue Culture Dish Size:</b>	24-well plate
<b>Expected Transfection Efficiency:</b>	>50% by GFP immunofluorescence

### Procedures

1. The day before transfection, plate the cells at  $5 \times 10^5$  cells per well in 200  $\mu$ l of serum-containing culture medium.
2. For each well, dilute 10  $\mu$ l of GenePORTER<sup>®</sup> 2 reagent with 40  $\mu$ l of OptiMem<sup>™</sup>.
3. For each well, dilute 2  $\mu$ g of DNA at 1  $\mu$ g/ $\mu$ l with 50  $\mu$ l of DNA Diluent.
4. Add the diluted DNA to the diluted GenePORTER<sup>®</sup> 2 reagent, mix well by pipetting several times and incubate at room temperature for 5-15 minutes.
5. Add the GenePORTER<sup>®</sup>2/DNA complexes directly to the cells growing serum-containing culture medium. The final transfection volume should now be 250  $\mu$ l for each well.
6. Incubate overnight under 5-10% CO<sub>2</sub> at 37°C.
7. Twenty-four hours post-transfection, feed the cells with 250  $\mu$ l of fresh growth media for each well.
8. Assay for gene expression 48 hours post-transfection.

Note: Transfection efficiencies may vary depending on the promoter, health of the cells, reporter gene, and method of assay.