

# **GeneSilencer® 96 siRNA Transfection Reagent**

## **Instruction Manual**

**GeneSilencer® 96 siRNA Transfection Reagent Titration Plate**  
**Catalog Numbers: T500960 (1 Plate) / T504960 (4 Plates)**

**GeneSilencer® 96 siRNA Transfection Reagent Standard Plate (High)**  
**Catalog Numbers: T500961 (1 Plate) / T504961 (4 Plates)**

**GeneSilencer® 96 siRNA Transfection Reagent Standard Plate (Low)**  
**Catalog Numbers: T500962 (1 Plate) / T504962 (4 Plates)**



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## Purchaser Notification

### Limited License

The purchase price paid for the GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended **for internal research only** by the purchaser. Such use is limited to the transfection of nucleic acids as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from 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”).

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The GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

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## OVERVIEW

### Kit Contents

Catalogue Number	Description	Content
T500960	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Titration Plate	1 x 96-well plate containing 11.7 µl to 0.15 µl of GeneSilencer <sup>®</sup> Reagent per well 1 x 0.5 ml siRNA Diluent
T504960	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Titration Plate	4 x 96-well plates containing 11.7 µl to 0.15 µl of GeneSilencer <sup>®</sup> Reagent per well 4 x 0.5 ml siRNA Diluent
T500961	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (High)	1 x 96-well plate containing 3.5 µl of GeneSilencer <sup>®</sup> Reagent per well 1 x 0.5 ml siRNA Diluent
T504961	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (High)	4 x 96-well plates containing 3.5 µl of GeneSilencer <sup>®</sup> Reagent per well 4 x 0.5 ml siRNA Diluent
T500962	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (Low)	1 x 96-well plate containing 0.7 µl of GeneSilencer <sup>®</sup> Reagent per well 1 x 0.5 ml siRNA Diluent
T504962	GeneSilencer <sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (Low)	4 x 96-well plates containing 0.7 µl of GeneSilencer <sup>®</sup> Reagent per well 4 x 0.5 ml siRNA Diluent

### Shipping and Storage

The GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Plates are shipped at room temperature. For maximum stability, store all reagents at 4°C upon receipt. **Keep plates sealed while in storage to prevent evaporation and/or contamination.** If stored properly, all components are stable for 6 months.

### Related Products

*For efficient generation of siRNA:*

Product Name	Cat. No.	Quantity
Dicer siRNA Generation Kit	T510001	5 genes / 50 transfections

*For efficient transfection of siRNA:*

Product Name	Cat. No.	Quantity
GeneSilencer <sup>®</sup> siRNA Transfection Reagent	T500750	200 reactions
GeneSilencer <sup>®</sup> siRNA Transfection Reagent	T505750	5 x 200 reactions

### Product Support

To place an order	To contact technical support
<b>Tel:</b> 858-457-1919, Ext. 2 OR 888-428-0558, Ext. 2 (US toll free) <b>Fax:</b> 858-623-9494 <b>Web:</b> <a href="http://www.genlantis.com">http://www.genlantis.com</a>  For a complete list of international distributors, please visit our website at: <a href="http://www.genlantis.com">www.genlantis.com</a>	<b>Tel:</b> 858-457-1919 Ext., 3 OR 888-428-0558 Ext., 3 (US toll free) <b>Fax:</b> 858-623-9494 <b>E-mail:</b> <a href="mailto:tech1@genlantis.com">tech1@genlantis.com</a>

## Introduction

GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Plates provide a convenient high-throughput format for optimizing transfection conditions and performing siRNA transfection in 96-well plates. All wells (except those in Column 12) contain a predetermined amount of the GeneSilencer<sup>™</sup> siRNA Transfection Reagent, a cationic lipid based reagent that has been extensively screened in many mammalian cell lines in order to achieve:

- Efficient delivery of siRNA
- Functional gene silencing post siRNA delivery
- Compatibility with diverse growth conditions (with and without serum)
- Low cytotoxicity

When compared to other commercially available transfection reagents, GeneSilencer<sup>™</sup> consistently offers superior transfection efficiencies and more effective gene silencing.

GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Plates are provided in 3 related configurations:

The **GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Titration Plate** contains serially diluted transfection reagent across the columns of the plate. Each well contains 11.7 - 0.15 µl of lipid diluted in serum-free media to a final volume of 25 µl. This format allows for the determination of the optimum amount of GeneSilencer<sup>®</sup> reagent needed for maximal siRNA delivery. Additionally, optimal siRNA quantity may be determined by the addition of decreasing amounts of siRNA down the rows of the plate.

The **GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (High)** contains the fixed amount of 3.5 µl of GeneSilencer<sup>®</sup> siRNA Transfection Reagent + 21.5 µl of serum free medium in each well of the plate. This allows for rapid transfections in a 96-well format when a higher optimal volume of GeneSilencer<sup>®</sup> has been established using the Titration Plate or from previous experiments or data.

The **GeneSilencer<sup>®</sup> 96 siRNA Transfection Reagent Standard Plate (Low)** contains the fixed amount of 0.7 µl of GeneSilencer<sup>®</sup> siRNA Transfection Reagent + 24.3 µl of serum free medium in each well of the plate. This allows for rapid transfections in a 96-well format when a lower optimal volume of GeneSilencer<sup>®</sup> has been established using the Titration Plate or from previous experiments or data.

## METHODS AND PROCEDURES

### 1. GeneSilencer® 96 siRNA Transfection Reagent Titration Plate

- 1.1. The day before transfection split the cells so they are in good condition on the day of transfection.
- 1.2. Review the pre-aliquoted GeneSilencer® amounts in the Titration Plate Layout (Table 1). Establish a range of siRNA amounts to dispense in each row of the Plate, e.g. 20 ng – 240 ng.

**Table 1: Titration Plate Layout**

		GeneSilencer® Reagent Amount Per Well (µl)													
		117	78	52	35	23	15	10	07	05	03	015	0		
Row Number	A	○	○	○	○	○	○	○	○	○	○	○	○	A	Row Number
	B	○	○	○	○	○	○	○	○	○	○	○	○	B	
	C	○	○	○	○	○	○	○	○	○	○	○	○	C	
	D	○	○	○	○	○	○	○	○	○	○	○	○	D	
	E	○	○	○	○	○	○	○	○	○	○	○	○	E	
	F	○	○	○	○	○	○	○	○	○	○	○	○	F	
	G	○	○	○	○	○	○	○	○	○	○	○	○	G	
	H	○	○	○	○	○	○	○	○	○	○	○	○	H	
		1	2	3	4	5	6	7	8	9	10	11	12		
		Column Number													

- 1.3. Centrifuge the Titration Plate in a swinging platform rotor at 2,000 to 3,000 rpm (~1000 x g) for at least 1 minute so that the 25 µl of diluted GeneSilencer® reagent will be collected at the bottom of each well. Peel away the adhesive seal by holding the plate firmly on a flat surface and peeling away the seal gently using the white tabs on either end.
- 1.4. If using adherent cells, detach cells from well surface by adding 5% trypsin/EDTA and incubating approximately 3 minutes. Inactivate the trypsin by adding an equal volume of 20% serum-containing medium to the well. If using suspension cells, no trypsinization step is necessary.
- 1.5. Spin down the cells and resuspend to the recommended number per well (see Table 2, p. 8) in a final volume of 40 µl of culture medium.

**TIP**

*For most cell types, we recommend using 10% serum-containing media in step 1.5. However, for some cell lines like HeLa, MDCK, and CHO-K1, transfection efficiencies may be higher if serum is omitted from the medium at this step until 4 hours after the start of transfection. Subsequently, add one volume of medium containing 20% serum, then proceed to step 1.9.*

**Table 2: Recommend Seeding Numbers for Common Cell Types in a 96-Well Plate**

Cell Type	Cells/Well	Cell Type	Cells/Well
B16-F0	10,000	HeLa	35,000
BHK-21	10,000	HepG2	25,000
CHO-K1	15,000	K562	75,000
COS-1	12,000	MDCK	8,000
COS-7	10,000	NIH-3T3	12,000
CV-1	12,000	PC-12	100,000
HEK-293	25,000		

- 1.6. Prepare your siRNA by mixing it with a combination of the siRNA Diluent (provided) and serum free medium (SFM) to make a total volume of 35  $\mu$ l. First, mix the siRNA Diluent with SFM. Then use the Diluent/SFM mix to dilute your siRNA. Use 1  $\mu$ l of siRNA Diluent per 20 ng of siRNA. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.

**NOTES**

1. We recommend you make a master mix of siRNA Diluent + SFM + your siRNA.
2. Avoid vortexing the siRNA/Diluent mix.

- 1.7. Add the 35  $\mu$ l of diluted siRNA solution from Step 1.6 above to the 25  $\mu$ l of GeneSilencer™ solution contained in each well of the Incubation Plate. Incubate at Room Temperature for 5 min. to allow the siRNA/lipid complexes to form.

**TIP**

*You can incubate the siRNA/GeneSilencer® mix for longer than 5 minutes, but make sure not to exceed 30 minutes in order to maintain maximum siRNA transfection efficiency.*

- 1.8. Add the 40  $\mu$ l of cells to the siRNA/GeneSilencer® complexes in Titration Plate. The total transfection volume should now be 100  $\mu$ l. Mix well by pipetting up and down several times. Incubate at 37°C for 24 hours.

**NOTE**

The morphology of adherent cells appears less healthy with this procedure than if the siRNA/GeneSilencer® complexes were added to seeded cells. However, we have found that transfection efficiency is actually enhanced when detaching the cells before transfection.

- 1.9. Add fresh tissue culture medium to growing cells as needed. Most RNA interference can be detected within 24 to 72 hours post transfection.

**2. GeneSilencer® 96 siRNA Transfection Reagent Standard Plate (Both High and Low)**

- 2.1 The day before transfection split the cells so they are in good condition on the day of transfection.
- 2.2 Centrifuge the Standard Plate in a swinging platform rotor at 2,000 to 3,000 rpm (~1000 x g) for at least 1 minute so that the 25  $\mu$ l of diluted GeneSilencer® reagent will be collected at the bottom of each well. Peel away the adhesive seal by holding the plate firmly on a flat surface and peeling away the seal gently using the white tabs on either end.
- 2.3 If using adherent cells, detach from well surface by adding 5% trypsin/EDTA and incubating approximately 3 minutes. Inactivate the trypsin by adding an equal volume of 20% serum-containing medium to the well. If using suspension cells, no trypsinization step is necessary.

- 2.4 Spin down the cells and resuspend to the recommended number per well (see Table 2) in a final volume of 40 µl of culture medium.

**TIP**

*For most cell types, we recommend using 10% serum-containing media in step 2.4. However, for some cell lines like HeLa, MDCK, and CHO-K1, transfection efficiencies may be higher if serum is omitted from the medium at this step until 4 hours after the start of transfection. Subsequently, add one volume of medium containing 20% serum, then proceed to step 2.9.*

**Table 2: Recommend Seeding Numbers for Common Cell Types in a 96-Well Plate**

Cell Type	Cells/Well	Cell Type	Cells/Well
B16-F0	10,000	HeLa	35,000
BHK-21	10,000	HepG2	25,000
CHO-K1	15,000	K562	75,000
COS-1	12,000	MDCK	8,000
COS-7	10,000	NIH-3T3	12,000
CV-1	12,000	PC-12	100,000
HEK-293	25,000		

- 2.5 Based on the optimum ratio of siRNA:GeneSilencer<sup>®</sup> Reagent determined from the Titration Plate, calculate how much siRNA is needed per well based on 0.5 µl of GeneSilencer<sup>®</sup> Reagent per well.
- 2.6 Prepare your siRNA by mixing it with a combination of the siRNA Diluent (provided) and serum free medium (SFM) to make a total volume of 35 µl. First, mix the siRNA Diluent with SFM. Then use the Diluent/SFM mix to dilute your siRNA. Use 1 µl of siRNA Diluent per 20 ng of siRNA. Mix well by pipetting up and down several times. Incubate at Room Temperature for 5 minutes.

**NOTES**

- We recommend you make a master mix of siRNA Diluent + SFM + your siRNA*
- Avoid vortexing the siRNA/Diluent mix.*

- 2.7 Add the 35 µl of diluted siRNA solution from Step 2.6 above to the 25 µl of GeneSilencer<sup>®</sup> solution contained in each well of the Plate. Incubate at Room Temperature for 5 minutes to allow the siRNA/lipid complexes to form.

**TIP**

*You can incubate the siRNA/GeneSilencer<sup>®</sup> mix for longer than 5 minutes, but make sure not to exceed 30 minutes in order to maintain maximum siRNA transfection efficiency.*

- 2.8 Add the 40 µl of cells to the siRNA/GeneSilencer<sup>®</sup> complexes in Titration Plate. The total transfection volume should now be 100 µl. Mix well by pipetting up and down several times. Incubate at 37°C for 24 hours.

**NOTE**

The morphology of adherent cells appears less healthy with this procedure than if the siRNA/GeneSilencer<sup>®</sup> complexes were added to seeded cells. However, we have found that transfection efficiency is actually enhanced when detaching the cells before transfection.

- 2.9 Add fresh tissue culture medium to growing cells as needed. Most RNA interference can be detected within 24 to 72 hours post transfection.

## APPENDIX

### Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low transfection efficiency	Suboptimal siRNA concentration.	When establishing the optimal GeneSilencer <sup>®</sup> /siRNA ratio using the Titration Plate, vary the siRNA quantity over the ranges suggested in Table 1.
	Poor siRNA quality	Use RNase-free handling procedures and plasticware. siRNA should be gel-purified. Check for degradation of siRNA on acrylamide gels.
	Denatured siRNA	Use recommended buffer (100 mM NaCl, 50 mM Tris, pH 7.5 in RNase-free water) to dilute siRNA. Do not use water as it can denature the siRNA.
	Cells have been in continuous passage for more than 2 months	Thaw out a fresh aliquot of cells and passage once (or more) before transfecting. Avoid using cells that have been in culture or have been passaged for excessive periods of time.
	Suboptimal cell density.	Use cells that are 50-70% confluent on the day of transfection. Optimal cell density may vary depending on cell type.
	Improper storage.	GeneSilencer reagent is very stable but long exposure to elevated temperatures and/or excessive freeze/thaw cycles may cause degradation of the reagent. Store GeneSilencer <sup>™</sup> reagent at 4° C.
	Wrong medium.	Be sure to use serum-free medium when forming the GeneSilencer <sup>®</sup> /siRNA complex.
	Cell line is difficult to transfect.	Optimize GeneSilencer <sup>®</sup> /siRNA ratio and siRNA amount as indicated in Appendix.
Aggregation	GeneSilencer <sup>®</sup> /siRNA complexes not freshly prepared.	GeneSilencer <sup>®</sup> /siRNA complexes should be freshly prepared. If complexes have been prepared and stored for longer than 45 minutes, aggregation may occur.
	Suboptimal GeneSilencer <sup>®</sup> /siRNA ratio used.	Too much GeneSilencer <sup>®</sup> or too much siRNA could cause aggregation. Test the ratios suggested in Table 1.
Cytotoxicity	GeneSilencer <sup>®</sup> concentration too high relative to cell numbers.	Increase cell numbers in 10-20% increments.
	Unhealthy cells.	<ul style="list-style-type: none"> <li>- Check cells for contamination.</li> <li>- Thaw a new batch of cells.</li> <li>- Cells are too confluent or cell density too low.</li> <li>- Check culture medium (pH, kind used, last time changed).</li> <li>- Check materials used for proper function (culture plates, incubator temperatures, etc.).</li> </ul>

For additional troubleshooting assistance, please contact our Technical Support Department:

<b>Tel:</b> 858-457-1919 Ext., 3 OR 888-428-0558 Ext., 3 (US toll free) <b>Fax:</b> 858-623-9494	<b>E-mail:</b> <a href="mailto:tech1@genlantis.com">tech1@genlantis.com</a> <b>URL:</b> <a href="http://www.genlantis.com">www.genlantis.com</a>
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