GeneSilencer®

siRNA Transfection Reagent

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Content	Amount
T500020	GeneSilencer Transfection Reagent	1 x 0.2 ml
	siRNA Diluent	1 x 1.0 ml
T500750 (~200 Rxns.)	GeneSilencer Transfection Reagent	1 x 0.75 ml
	siRNA Diluent	1 x 4 ml
T505750 (~1000 Rxns.)	GeneSilencer Transfection Reagent	5 x 0.75 ml
	siRNA Diluent	5 x 4ml

Shipping	Shipped at room temperature.
Storage	Store at 4°C; stable for 1 year at 4°C.

RELATED PRODUCTS	Catalog Numbers
GeneSilencer 96 Titration Plate	T500960 (1 plate); T504960 (4 plates)
GeneSilencer 96 Standard Plate, High	T500961 (1 plate); T504961 (4 plates)
GeneSilencer 96 Standard Plate, Low	T500962 (1 plate); T504962 (4 plates)
GeneSilencer shRNA Vector Kits	P100100 (H1); P100300 (H1-GFP)
	P600100 (U6); P600300 (U6-GFP)
GeneSilencer PCR Kits	P140100 (H1); P640100 (H1-GFP)
	P140300 (U6); P640300 (U6-GFP)
Turbo Dicer siRNA Generation Kit	T520001
Turbo Dicer Enzyme Kit	T520002
siGuard RNase Inhibitor	T520100

INTRODUCTION

GeneSilencer® siRNA Transfection Reagent is a unique cationic lipid formulation specifically designed for efficient delivery of siRNAs (small interfering RNAs) into a wide variety of cell types. GeneSilencer is easy to use, exhibits low cytotoxicity compared to competitor reagents, and works efficiently with both adherent and suspension cells. Table 1 across shows some popular cells lines that have been successfully transfected with GeneSilencer (visit www.genlantis.com for an updated list of published citations).

Table 1: Cell Lines Successfully Transfected with GeneSilencer

Cell Line	Best In	Cell Line	Best In
HeLa or HeLa-S3	SF	B15-F0	S
COS-1	S	293	S
COS-7	S	BHK-21	S
Hep-G2	S	CHO-K1	SF
NIH-3T3	S	PC-12	S
MDCK	SF	P19	S
K-562	SF	MCF-7	SF
CV-1	S	Neuro2a	SF
Jurkat	SF	HUVEC-C	S
S = With serum; SF = Serum-free			
For an updated list of cells & citations, visit www.genlantis.com			

MATERIALS AND METHODS

A. Transfection of Adherent Cells

1. On the day BEFORE transfection, plate cells so they will be 50-70% confluent on transfection day; use recommended media volumes per well in Table 2:

   Table 2: Cell Seeding Media Volumes Per Well.

Plate Type	Media Volume per Well (µl)
96 well	100
48 well	200
24 well	500
6 well	1,000

2. On transfection day, dilute GeneSilencer® by adding the indicated volume to the serum-free medium volume shown in Table 3:

   Table 3:GeneSilencer Dilutions For Adherent Cells.

Plate Type	GeneSilencer Per Well (µl)	Serum Free Medium Per Well (µl)
96 well	1.0	25
48 well	1.75	25
24 well	3.5	25
6 well	5.0	25

3. Dilute the supplied siRNA Diluent with the appropriate volume of serum free media as indicated in Table 4. Then, add the quantity of siRNA indicated in Table 4 to the siRNA Diluent/serum-free media mixture.

   Table 4: Dilution of siRNA For Adherent Cells.

Plate Type	siRNA Diluent Per Well (µl)	Serum Free Medium/Well (µl)	siRNA Per well (ng)
96 well	2.5	15.0	50
48 well	5.0	15.0	100
24 well	10.0	15.0	200
6 well	25.0	15.0	1,000

4. Incubate the diluted siRNA mixture at room temperature for 5 min.
   Important: do NOT vortex the diluted siRNA mix.

5. Prepare the Transfection Mix by combining the diluted GeneSilencer from Step 2 with the siRNA mixture from Step 4. Incubate at room temp for 5-30 min. (5 min. is typically sufficient).
   Important: do NOT incubate longer than 30 minutes.

6. For cell lines that grow best in serum containing media (see Table 1):
   a. Add the Transfection Mix from Step 5 directly to plated cells.
   b. Incubate at 37°C for 24 to 72 hours; assay for gene silencing.

7. For cell lines that grow best in serum-free media (see Table 1):
   a. Remove media containing serum from cells prepared in Step 1.
   b. Replace serum media with same volume of serum-free media.
   c. Add the Transfection Mix from Step 5 to the cells.
   d. Incubate at 37°C for 4 hours.
   e. Add 1 volume of media containing 20% serum.
   f. Incubate at 37°C for 24 to 72 hours; assay for gene silencing.

B. Transfection of Suspension Cells

8. On the day BEFORE transfection, split the cells to optimize their health and achieve log phase growth at time of transfection.

9. On transfection day, dilute GeneSilencer® by adding the indicated volume to the serum-free medium volume shown in Table 5:

Table 5:GeneSilencer Dilution for Suspension Cells.

Plate Type	GeneSilencer Per Well (µl)	Serum Free Medium Per Well (µl)
96 well	1.0	25
48 well	1.75	25
24 well	3.5	25
6 well	5.0	25

10. Dilute the supplied siRNA Diluent with the appropriate serum free media volume indicated in Table 6. Then, add the quantity of siRNA indicated in Table 6 to the siRNA Diluent/serum-free media. **Note:** For Jurkat cells, substitute siRNA Diluent with serum-free media

Table 6: siRNA Dilution for Suspension Cells.

Plate Type	siRNA Per well (ng)	siRNA Diluent Per Well (µl)	Serum Free Medium/Well (µl)
96 well	50	2.5	15.0
48 well	100	5.0	15.0
24 well	200	10.0	15.0
6 well	1,000	25.0	15.0

11. Incubate the diluted siRNA mixture at room temperature for 5 min. **Important**: do NOT vortex the diluted siRNA mixture.

12. Prepare the Transfection Mix by combining the diluted GeneSilencer from Step 9 with the diluted siRNA mix from Step 11. Incubate at room temperature for 5-30 minutes (5 minutes is typically sufficient). **Important**: do NOT incubate longer than 30 minutes.

13. While the Transfection Mix (from Step 12) is incubating, spin down suspension cells and gently remove the culture medium.

14. Resuspend cells in serum or serum-free medium (based on type of cells used) and transfer cells to wells as recommended in Table 7:

Table 7: Volume and Number of Suspension Cells Per Plate Type.

Plate Type	Resuspended Cell Volume Per Well (µl)	Cells Number Per Well
96 well	100	1×10^5
48 well	200	2×10^5
24 well	500	5×10^5
6 well	1000	2×10^6

15. Add the Transfection Mix (from Step 12) to the resuspended cells in each of the plate wells. Table 8 below shows what the approximate final volumes per well should be:

Table 8: Summary of Transfection Mix Volumes Per Well

Plate Type	Diluted GeneSilencer (Table 5)	Diluted siRNA (Table 6)	Cell Volume (Table 7)	Final Transfection Volume
96 well	26 µl	17.5 µl	100 µl	143.5 µl
48 well	26.75 µl	20.0 µl	200 µl	246.75 µl
24 well	28.5 µl	35.0 µl	500 µl	563.5 µl
6 well	30.0 µl	40.0 µl	1000 µl	1,070.0 µl

16. After adding the Transfection Mix to the cells, gently mix by pipetting up and down several times; this is important to avoid cell clumping.

17. If using serum-free medium for transfection, add one volume of 2X serum-containing medium after 4 hours

18. Incubate the transfected cells at 37°C for 24 to 72 hours (to detect RNA interference) and periodically add fresh tissue culture medium to cells as needed.

TROUBLESHOOTING - [GS = GeneSilencer® Transfection Reagent]

Problem	Possible Causes	Solution
Low Transfection Efficiency	CRITICAL: Suboptimal GS:siRNA ratio	Optimize GS:siRNA ratio: use 0.5-7.0 ml GS per 100 ng of siRNA. Use a low siRNA quantity to optimize this parameter. Optionally, you can use the GeneSilencer Titration Plate (Cat # T500960) for additional and convenient optimization.
	CRITICAL: Suboptimal siRNA concentration	After establishing optimal GS/siRNA ratio, vary the siRNA quantity over the ranges suggested in Materials and Methods. Optionally, you can use the GeneSilencer Titration Plate (Cat # T500960) for additional and convenient optimization.
	Poor siRNA quality	Use RNase-free plastic ware and procedures; gel-purify siRNA and check on acrylamide gel. Use siGuard RNase Inhibitor.
	Denatured siRNA	Use recommended buffer (100mM NaCl, 50mM Tris pH7.5 in RNase-free water) to dilute siRNA; water only denatures siRNA.
	Cells too old	Use freshly thawed cells and passage only a few times before transfecting. Avoid cells that have been excessively passaged.
	Cell density	Use cells that are 50-70% confluent on day of transfection. Optimal cell density may vary depending on cell type.
	GS degraded	GS reagent is very stable but extreme conditions may cause degradation. Try a new lot or batch of GS for testing.
	Wrong medium	Make sure to use serum-free medium when forming the Transfection Mix (Steps 5 and 12).
	Cell line used	Some cells are difficult to transfect; try different cells or optimize GS:siRNA ratio and siRNA amounts as recommended above.
	Transfection Mix not freshly prepared	The Transfection Mix (i.e. diluted GeneSilencer plus the diluted siRNA mixture) should be freshly prepared. If Transfection Mix has been prepared and stored for longer than 45 minutes, aggregation may occur.
Aggregation	Excess GS used	Use less GeneSilencer Reagent and also lower the amount of siRNA used to keep GS:siRNA ratio optimized.
Cytotoxicity	Unhealthy cells or faulty equipment	Check for contaminated cells; thaw a new batch of cells; make sure cell densities are not too low or too high; use the recommended culture medium and check pH if suspicious; supply cells with fresh medium at regular intervals; check equipment for malfunctions.
	[GS] too high	Use less GeneSilencer Reagent and also lower the amount of siRNA used to keep GS:siRNA ratio optimized.