# GeneSilencer®

amsbio Accelerate Your Discovery

## siRNA Transfection Reagent

| Catalog # | Content | Amount |
|---|---|---|
| AMS.T500020 (50 Rxns.) | GeneSilencer Transfection Reagent | 1 x 0.2 ml |
| | siRNA Diluent | 1 x 1.0 ml |
| AMS.T500750 (~200 Rxns.) | GeneSilencer Transfection Reagent | 1 x 0.75 ml |
| | siRNA Diluent | 1 x 4 ml |
| AMS.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. |

### 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.

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

### 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 cells that transfect and grow best in media with serum (Table 1):
   a. Add the Transfection Mix from Step 5 directly to plated cells.
   b. Incubate at 37C for 24 to 72 hours; assay for gene silencing.
7. For cells that grow best in media with serum but transfect best in serum-free media (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 37C for 4 hours.
   e. Add 1 volume of media containing 20% serum.
   f. Incubate at 37C for 24 to 72 hours; assay for gene silencing
8. For cells that transfect and grow best in serum-free media (Table 1):
   a. Add the Transfection Mix from Step 5 directly to plated cells.
   b. Incubate at 37C for 24 to 72 hours; assay for gene silencing



**AMSBIO LLC**
USA & Canada
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

**AMSBIO Europe BV**
EU
Berenkoog 41,
1822 BH Alkmaar,
Netherlands
T: +31 (0) 72 8080244
F: +31 (0) 72 8080142

**AMS Biotechnology (Europe) Ltd**
UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

**AMS Biotechnology (Europe) Ltd**
Switzerland
Via Lisano 3,
(CP.683)
CH-6900 Massagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

**AMSBIO Europe BV**
Deutschland
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

## B. Transfection of Suspension Cells

1. On the day BEFORE transfection, split the cells to optimize their health and achieve log phase growth at time of transfection.
2. 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 |

3. 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 |

4. Incubate the diluted siRNA mixture at room temperature for 5 min. Important: do NOT vortex the diluted siRNA mixture.
5. Prepare the Transfection Mix by combining the diluted GeneSilencer from Step 10 with the diluted siRNA mix from step 2. Incubate at room temperature for 5-30 minutes (5 minutes is typically sufficient). Important: do NOT incubate longer than 30 minutes.
6. While the Transfection Mix (from Step 13) is incubating, spin down suspension cells and gently remove the culture medium.
7. 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 | $1 \times 10^5$ |
| 48 well | 200 | $2 \times 10^5$ |
| 24 well | 500 | $5 \times 10^5$ |
| 6 well | 1000 | $2 \times 10^6$ |

8. Add the Transfection Mix (from Step 13) 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 |

9. After adding the Transfection Mix to the cells, gently mix by pipetting up and down several times; this is important to avoid cell clumping.
10. If using serum-free medium for transfection, add one volume of 2X serum-containing medium after 4 hours
11. 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.



**AMSBIO LLC**
**USA & Canada**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

**AMSBIO Europe BV**
**EU**
Berenkoog 41,
1822 BH Alkmaar,
Netherlands
T: +31 (0) 72 8080244
F: +31 (0) 72 8080142

**AMS Biotechnology (Europe) Ltd**
**UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

**AMS Biotechnology (Europe) Ltd**
**Switzerland**
Via Lisano 3,
(CP.683)
CH-6900 Massagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

**AMSBIO Europe BV**
**Deutschland**
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

**TROUBLESHOOTING - [GS = GeneSilencer® Transfection Reagent]**

| Problem | Possible Causes | Solution |
|---|---|---|
| Low Transfection Efficiency | CRITICAL: Suboptimal GS:siRNA ratio | Optimize GS:siRNA ratio: use 0.5-3.0 µl 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. |



**AMSBIO LLC**
**USA & Canada**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

**AMSBIO Europe BV**
**EU**
Berenkoog 41,
1822 BH Alkmaar,
Netherlands
T: +31 (0) 72 8080244
F: +31 (0) 72 8080142

**AMS Biotechnology (Europe) Ltd**
**UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

**AMS Biotechnology (Europe) Ltd**
**Switzerland**
Via Lisano 3,
(CP.683)
CH-6900 Massagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

**AMSBIO Europe BV**
**Deutschland**
T: +49 (0) 69 779099
F: +49 (0) 69 13376880