GeneGlide™ siRNA Transfection Reagent

(Catalog # M1081-300, -500, -1000; Store at 4°C)

I. Introduction:

BioVision's GeneGlide™ siRNA Transfection reagent is a cationic proprietary polymer/lipid formulation, and is non-liposomal. It is a broad spectrum siRNA transfection reagent that enables high efficiency siRNA delivery and knockdown of target gene expression in many cell types including primary cells. Co-transfection of siRNA and DNA is also feasible with GeneGlide™ siRNA Transfection reagent has been tested across a variety of cell types. Transfections with GeneGlide™ siRNA Transfection reagent do not require medium changes and can be carried out in serum-containing medium. The unique formulation provides high efficiency broad-spectrum siRNA delivery. GeneGlide™ siRNA Transfection reagent can be used to transfect two different siRNA duplexes at the same time. No adjustment in the amount of GeneGlide™ reagent per well is needed. However, the total concentration of siRNAs should be maintained between 10-50 nM.

II. Sample Type:

All mammalian cell types typically hard-to-transfect cells including primary cells and cell lines.

III. Package Contents:

M1081-300	1 X 300 µl
M1081-500	1 X 500 µl
M1081-1000	1 X 1000 µl

IV. User Supplied Reagents and Equipment:

- Cultured cells
- Appropriate cell culture medium
- Purified plasmid DNA
- Serum-free medium (e.g. Opti-MEM® I Reduced-Serum Medium)
- Sterile tube for transfection complex preparation
- Micropipettes
- Reporter assay as required

V. Shipment and Storage:

All the reagents are shipped on blue ice. Store tightly capped at 4˚C to prevent evaporation. The guaranteed shelf life is 12 months from the date of purchase when properly stored and handled.

VI. Reagent Preparation and Storage Conditions:

- Before each use, warm the reagent to room temperature and vortex gently.
- Do not use water alone to dilute siRNA, as this may result in denaturation of the siRNA duplex, especially at low concentrations. siRNA can also be diluted in annealing buffer that is supplied with your siRNA or Use 100 mM NaCl in 50 mM Tris , pH 7.5, made with RNase-free water.

VII. BEFORE YOU START:

Important Tips for Optimal siRNA Transfection:

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency transfection using BioVision's GeneGlide™ siRNA Transfection reagent. Table 1 presents recommended starting conditions depending on culture vessel size.

- **Cell density (% confluence) at transfection**
  Determine the optimal cell density for each cell type to maximize transfection efficiency. Divide the cells 18–24 hr before transfection to ensure that the cells are actively dividing and reach the appropriate cell density (generally ≥80% confluence) at the time of transfection. If this confluence does not produce optimal results, test cell densities outside of the recommended range.
- **Volume of GeneGlide™ siRNA Transfection reagent**
  Each cell type responds differently to a given transfection reagent. As a starting point, test 2.5 µl of GeneGlide™ siRNA Transfection reagent per well of a 24-well plate. For further optimization, test three levels of GeneGlide™ siRNA Transfection reagent, e.g. 1 µl, 2.5 µl, and 4 µl per well of a 24-well plate.
- **siRNA dilution**
  Dilute siRNA using the manufacturer's recommended buffer. Alternatively, use 100 mM NaCl in 50 mM Tris, pH 7.5, made with RNase-free water. Do not use water alone to dilute siRNA, as this may result in denaturation of the siRNA.
- **siRNA concentration**
  siRNA used for transfection should be highly pure, sterile, and the correct sequence. Depending on the type of experiment, the optimal final siRNA concentration for transfection is typically within the range of 10–50 nM. As a starting point, we recommend 25 nM siRNA (final concentration in well).
- **Proper controls**
  BioVision recommends transfecting a non-targeting or nonsense siRNA control sequence to verify that the gene expression knockdown or phenotype is attributed to the gene specific siRNA. Additionally, targeting a gene with multiple siRNA sequences ensures that the resulting phenotype is not due to off-target effects.
- **Complex formation conditions**

Prepare GeneGlide™ siRNA Transfection reagent:siRNA complexes in serum free growth medium. BioVision recommends Opti-MEM I Reduced-Serum Medium.

- **Cell culture conditions**
- Culture cells in the appropriate medium, with or without serum. There is no need to perform a medium change to remove the transfection complexes. The GeneGlide™ siRNA Transfection reagent yields improved transfection efficiencies when transfections are performed in complete growth medium (instead of serum-free medium) without a post-transfection medium change.
- **Presence of antibiotics**
  Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing low levels of antibiotics (0.1–1X final concentration of penicillin/streptomycin mixture).
- **Transfection incubation time**
  The optimal incubation time can be determined empirically by testing a range from 24–72 hr post-transfection, depending on the stability of the target mRNA and its encoded protein. When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hr post-transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post-transfection incubation may be necessary particularly if the target protein has a long cellular half-life.

Additional Tips for DNA and siRNA Co-Transfection:

Observe the following recommendations in addition to the tips for siRNA transfection when performing a co-transfection of DNA and siRNA. This procedure is further outlined in the DNA and siRNA Co-Transfection Protocol. The suggestions below yield high efficiency knockdown of target gene expression using GeneGlide™ siRNA Transfection reagent for DNA and siRNA delivery.

- **Cell density (% confluence) at transfection.** The recommended cell density for most cell types is ≥80% confluence. Determine the optimal cell density for each cell type in order to maximize transfection efficiency. Divide the cells 18–24 hr before transfection to ensure that the cells are actively dividing and reach the appropriate cell density at the time of transfection.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preps that are endotoxin-free and have A260/280 absorbance ratio of 1.8–2.0 are desirable. DNA prepared using miniprep kits is not recommended as it might contain high levels of endotoxin. We recommend removing any traces of endotoxin from your DNA preparation.
- **Ratio of GeneGlide™ siRNA Transfection reagent to DNA.** Determine the best GeneGlide™ siRNA Transfection reagent:DNA ratio for each cell type. Start with 1 µl of GeneGlide™ siRNA Transfection reagent per 1 µg of DNA. Vary the concentration of GeneGlide™ siRNA Transfection reagent from 2–8 µl per 1 µg DNA to find the optimal ratio. Table 2 provides recommended starting conditions based on cell culture vessel size.
- **Proper controls.** BioVision recommends transfecting a plasmid only control to verify gene expression and provide a reference for determining gene expression knockdown.
- **Complex formation conditions.** Prepare GeneGlide™ siRNA Transfection reagent:DNA complexes in serum free growth medium. BioVision recommends Opti-MEM I Reduced-Serum Medium.
- **Cell culture conditions.** Culture cells in the appropriate medium. The GeneGlide™ siRNA Transfection reagent yields improved efficiencies when transfections are performed in complete growth medium without a post-transfection medium change. There is no need to perform a medium change to remove the transfection complexes.
- **Presence of antibiotics.** Antibiotics will inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added to cells grown in complete culture medium containing serum and low levels of antibiotics (0.1-1X final concentration of penicillin/streptomycin mixture).
- **Post-transfection incubation time.** Determine the best incubation time post-transfection for each cell type. The optimal incubation time is generally 24-72 hr, but will vary depending on the goal of the experiment, nature of the plasmid used, and the half-life of the expressed protein.

VIII. siRNA TRANSFECTION PROTOCOL:

The following procedure describes how to perform siRNA transfection using GeneGlide™ siRNA Transfection reagent in 24-well plates. The surface areas of other culture vessels are different and transfection must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, GeneGlide™ siRNA Transfection reagent, siRNA and complete culture medium based on the surface area of the cell culture vessel (please refer to Table 1).

Table 1: Recommended starting conditions for siRNA transfection with GeneGlide™ siRNA Transfection reagent.

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10-cm dish	T75 flask
Surface area	0.35 cm^2	1.0 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2	75 cm^2
Complete growth medium	92 µl	263 µl	0.5 ml	1.0 ml	2.5 ml	15.5 ml	19.7 ml
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
GeneGlide™ siRNA Transfection reagent	0.5 µl	1.3 µl	2.5 µl	5 µl	10 µl	77 µl	98 µl
siRNA (10 µM stock) 25 nM final	0.25 µl	0.7 µl	1.4 µl	2.8 µl	6.8 µl	42.5 µl	54 µl

Transient siRNA transfection protocol per well of a 24-well plate:

A. Plate cells

1. Approximately 18-24 hr before transfection, plate cells using the following guidelines. For most cell types, cultures should be ≥80% confluent at the time of transfection.

For adherent cells: Plate cells at a density of 0.8-3.0 × 10^5 cells/ml.
For suspension cells: Plate cells at a density of 2.5-5.0 × 10^5 cells/ml.

2. Incubate the cell cultures overnight.

B. Prepare GeneGlide™ siRNA Transfection reagent:siRNA complexes (Immediately before transfection)

1. Warm GeneGlide™ siRNA Transfection reagent to room temperature and vortex gently before using.
2. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.

3. Add 2.5 µl of GeneGlide™ siRNA Transfection reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the GeneGlide™ siRNA Transfection reagent.
4. Add 1.4 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
5. Incubate at room temperature for 15-30 min to allow sufficient time for complexes to form.

C. Distribute the complexes to cells in complete growth medium

1. Add the GeneGlide™ siRNA Transfection reagent:siRNA complexes (prepared in Step B) drop-wise to different areas of the wells.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the GeneGlide™ siRNA Transfection reagent:siRNA complexes.
3. Incubate for 24-72 hr or as required. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay for knockdown of target gene expression.

Note: When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hr post transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary, particularly if the target protein has a long cellular half-life.

IX. DNA & siRNA CO-TRANSFECTION PROTOCOL: Co-transfection of DNA and siRNA is possible with GeneGlide™ siRNA Transfection reagent across a variety of cell types. Delivery of DNA with GeneGlide™ siRNA Transfection reagent into your cell type should first be determined prior to attempting the co-transfection. The following procedure describes how to perform the co-transfection of DNA and siRNA using GeneGlide™ siRNA Transfection reagent to cells in 24-well plates. The surface areas of other culture vessels are different and transfection must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, GeneGlide™ siRNA Transfection reagent, siRNA, DNA and complete culture medium based on the surface area of the cell culture vessel (please refer to Table 2). It is highly recommended to first optimize DNA transfection efficiency with GeneGlide™ siRNA Transfection reagent in your particular cell type before attempting the co-transfection.

Table 2. Recommended starting conditions for DNA and siRNA transfections with GeneGlide™ siRNA Transfection reagent.

Culture vessel	96-well plate	48-well plate	24-well plate	12-well plate	6-well plate	10-cm dish	T75 flask
Surface area	0.35 cm^2	1.0 cm^2	1.9 cm^2	3.8 cm^2	9.6 cm^2	59 cm^2	75 cm^2
Complete growth medium	92 µl	263 µl	0.5 ml	1.0 ml	2.5 ml	15.5 ml	19.7 ml
DNA complex							
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
GeneGlide™ siRNA Transfection reagent	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
SiRNA complex							
Serum-free medium	9 µl	26 µl	50 µl	100 µl	250 µl	1.5 ml	1.9 ml
siRNA (10 µM stock) 25 nM final	1.55 µl	0.78 µl	1.5 µl	3.0 µl	43.8 µl	263 µl	340 µl
GeneGlide™ siRNA Transfection reagent	0.28 µl	1.3 µl	2.5 µl	5 µl	10 µl	77 µl	98 µl

Transient DNA and siRNA co-transfection protocol per well of a 24-well plate:

A. Plate cells

1. Approximately 18-24 hr before transfection, plate cells using the following guidelines. For most cell types, cultures should be ≥ 80% confluent at the time of transfection.

For adherent cells: Plate cells at a density of 0.8-3.0 × 10^5 cells/ml
For suspension cells: Plate cells at a density of 2.5-5.0 × 10^5 cells/ml

2. Incubate the cells overnight.

B. Prepare Complexes (Immediately before transfection) *Note: Complex formation of* GeneGlide™ siRNA Transfection reagent:*siRNA (Steps 1 & 2) should be synchronized to allow for simultaneous addition to cells.*

1. Prepare GeneGlide™ siRNA Transfection reagent:DNA complexes

a. Warm GeneGlide™ siRNA Transfection reagent to room temperature and vortex gently before using.
b. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
c. Add 0.5 µl of GeneGlide™ siRNA Transfection reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the GeneGlide™ siRNA Transfection reagent.
d. Add 0.5 µg (0.5 µl of 1 µg/µl stock) plasmid DNA. *Note: If transfecting more than one plasmid, mix the plasmids together in a microcentrifuge tube and incubate for 5-10 min at room temperature before adding to the diluted* GeneGlide™ siRNA Transfection reagent to avo*id preferential complex formation of either plasmid.*
e. Pipet gently to mix completely.
f. Incubate at room temperature for 15-30 min to allow sufficient time for complexes to form.

2. Prepare GeneGlide™ siRNA Transfection reagent:siRNA complexes

a. Warm GeneGlide™ siRNA Transfection reagent to room temperature and vortex gently before using.
b. Place 50 µl of Opti-MEM I Reduced-Serum Medium in a sterile tube.
c. Add 2.5 µl of GeneGlide™ siRNA Transfection reagent. Pipet gently to mix completely. For further optimization of your cell type, test additional levels of the GeneGlide™ siRNA Transfection reagent.
d. Add 1.5 µl of a 10 µM siRNA stock solution (25 nM final concentration per well). Pipet gently to mix completely.
e. Incubate at room temperature for 15-30 min to allow sufficient time for complexes to form.

3. Combine complexes

a. After GeneGlide™ siRNA Transfection reagent:DNA and GeneGlide™ siRNA Transfection reagent:siRNA complex formation, add the complexes together.
b. Pipet gently to mix completely.
c. Incubate at room temperature for 5 min.

C. Distribute the complex mixture to cells in complete growth medium

1. Add the co-transfection complexes (prepared in Step B) drop-wise to different areas of the wells.
2. Gently rock the culture vessel back-and-forth and from side-to-side to evenly distribute the co-transfection complexes.
3. Incubate for 24-72 hr or as required. It is not necessary to replace the complete growth medium with fresh medium.

4. Harvest cells and assay for knockdown of target gene expression. *Note: If preferred, DNA transfection can also be performed using GeneGlide™ DNA Transfection reagent while delivering siRNA with GeneGlide™ siRNA Transfection reagent.*

X. Figures and Data:

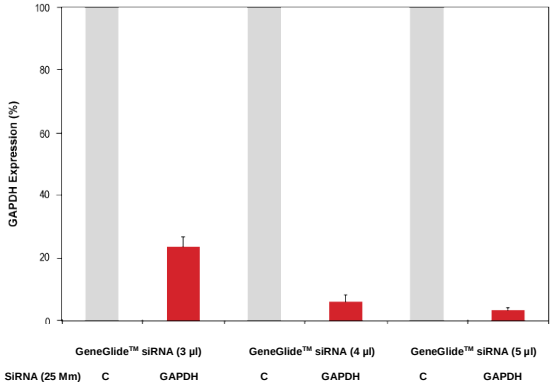

Figure 1. High Efficiency Endogenous Knockdown in iCell® Cardiomyocytes. The GeneGlide™ siRNA Transfection Reagent was used to transfect iCell® Cardiomyocytes (Cellular Dynamics International) plated at a density of 136,500 cells per well of a 12-well plate pre-coated with fibronectin. Seven days post-plating triplicate wells were transfected with GeneGlide™ siRNA (3-5 µl per well) and non-targeting control siRNA (C) GAPDH targeting siRNA (50nM per well). Seventy-two hr post-transfection, the amount of GAPDH mRNA was measured relative to 18s rRNA mRNA levels using qRT-PCR and then scaled to the expression level of the non-targeting control siRNA. Error bars represent the standard error of the mean (SEM) of three independent complexes.

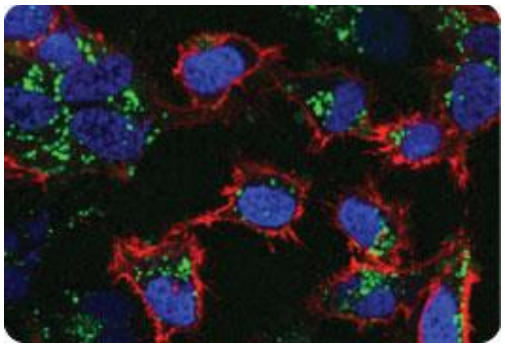

Figure 2. Delivery of Fluorescently-Labeled siRNA using GeneGlide™ siRNA Transfection Reagent. HeLa (70% confluence) cells in 12-well plates were transfected with GeneGlide™ siRNA Transfection Reagent (3 µl/well) and GeneGlide™ siRNA Tracker Fluorescein-labeled RNAi duplexes (GREEN, 50 nM final concentration in the well). The cells were incubated 24 hr post-transfection then fixed and counterstained with TO-PRO®-3 (nuclei, BLUE) (Life Technologies) and Alexa Fluor® 546 Phalloidin (actin, RED) (Life Technologies). Confocal images were acquired on a Zeiss LSM 510 Confocal Microscope.

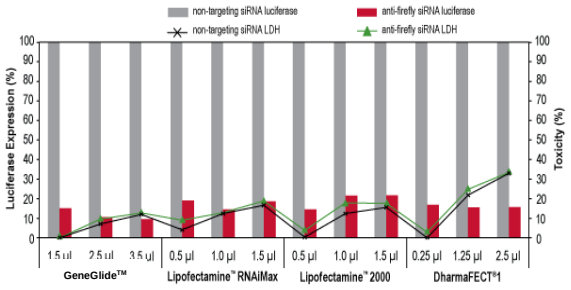

Figure 3. High Efficiency Knockdown and Low Toxicity Using GeneGlide™ siRNA Reagent in HeLa cells. Firefly and sea pansy luciferase reporter vectors were co-transfected into HeLa cells using GeneGlide™ siRNA Reagent. Cells were incubated for at least 4 hr, split into 24-well plates, allowed to adhere and transfected with 25 nM of either a non-targeting siRNA or an anti-firefly luciferase siRNA using the indicated reagents with the volumes noted beneath each well. Luciferase expression, normalized to non-targeting siRNA control (bar graph) and lactate dehydrogenase (LDH) levels (line graph) were measured at 24 hr post-transfection. LDH levels are reported as % cytotoxicity compared to cells alone and were measured using a commercially available colorimetric assay; all values at or below zero are represented as zero on graph.

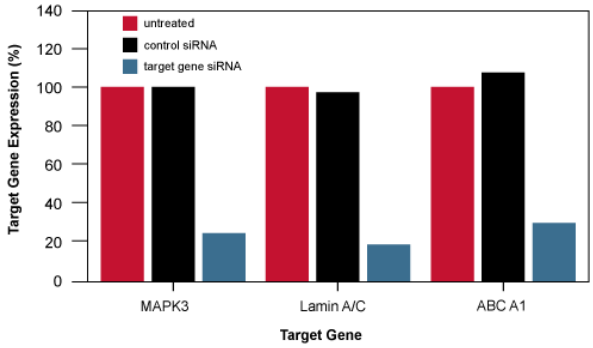

Figure 4. Efficient Knockdown of Endogenous Genes in Primary Hepatocytes Using GeneGlide™ siRNA Reagent. Primary mouse hepatocytes were transfected with the indicated siRNAs or a non-targeting control siRNA using the GeneGlide™ siRNA Reagent. 24 hr post-transfection, the amount of each mRNA was measured relative to GAPDH mRNA levels using qRT-PCR and then scaled to the expression level of the specific target mRNA in the cells alone (untreated) controls.

XI. Related Products:

Product Name	Catalog Number
GeneGlide™ DNA Transfection Reagent	M1080-300
GeneGlide™ DNA Transfection Reagent	M1080-500
GeneGlide™ DNA Transfection Reagent	M1080-1000
GeneGlide™ siRNATransfection Reagent	M1081-300
GeneGlide™ siRNA Transfection Reagent	M1081-500

GeneGlide™ siRNA Transfection Reagent	M1081-1000
GeneGlide™ RNAi Delivery Control	M1082-10
GeneGlide™ RNAi Delivery Control	M1082-50
GeneGlide™ RNAi Delivery Control	M1082-100

XII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
POOR siRNA KNOCK DOWN EFFICIENCY	• GeneGlide™ siRNA Transfection reagent was not mixed properly. • Suboptimal GeneGlide™ siRNA Transfection reagent:siRNA ratio. • Suboptimal siRNA concentration. • Proper controls were not included. • Denatured siRNA. • Incorrect siRNA Sequence. • Poor quality of siRNA. • Inhibitor present during transfection. • Transfection incubation time. • Cell-type dependence.	• Warm GeneGlide™ siRNA Transfection reagent to room temperature and vortex gently before each use. • For optimization, test three levels of GeneGlide™ siRNA Transfection reagent**,** e.g. 1, 2.5, and 4 µl per well of a 24-well plate, using 25 nM siRNA (final concentration in the well). It may be necessary to titrate outside of this range depending on the cell type. • Determine the optimal siRNA concentration by titrating from 10–50 nM (final concentration in the well). We recommend starting with 25 nM siRNA (final concentration in the well). In some instances, higher concentrations of siRNA up to 200 nM may be necessary to achieve sufficient knockdown of the gene of interest. • 1. Serum-free medium alone 2. Serum-free medium + GeneGlide™ siRNA Transfection reagent + a non-targeting siRNA. To verify efficient transfection and knockdown, use GeneGlide™ siRNA Transfection reagent to deliver a siRNA targeted against a ubiquitous gene, e.g. GAPDH or Lamin A/C, followed by target western blotting or mRNA quantification. • To assess delivery efficiency of siRNA, use RNAi Delivery Control. • To dilute siRNA, use the manufacturer's recommended buffer or 100 mM NaCl, 50 mM Tris, pH 7.5 in RNase-free water. Do not use water as this can denature the siRNA at low concentration during long-term storage. • Ensure that the sequence of the siRNA is correct for the gene of interest. More than one sequence may need to be tested for optimal knockdown efficiency and to ensure on-target effects. • Avoid siRNA degradation by using RNase-free handling procedures and plastic ware. Degradation of siRNA can be detected on acrylamide gels. • Serum and antibiotics inhibit transfection complex formation. Prepare all GeneGlide™ siRNA Transfection reagent complexes in serum-free growth medium. We recommend Opti-MEMI Reduced Serum medium. Once transfection complexes are formed, they can be added directly to cells cultured in complete growth medium containing serum and 0.1-1X antibiotics. The presence of polyanions e.g. dextran sulfate or heparin can inhibit transfection. Use transfection medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hr post transfection. • Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g. 24-72 hr). When quantifying knockdown efficiencies at the mRNA level, assaying at 24 hr post transfection is often sufficient. When quantifying knockdown efficiencies at the protein level, longer post transfection incubation may be necessary if the target protein has a long cellular half-life.
POOR DNA TRANSFECTION EFFICIENCY (FOR CO-TRANSFECTION)	• Incorrect vector sequence. • Transfection incubation time. • Cells not actively dividing at the time of transfection.	• If you do not observe expression of your target insert, verify the sequence of the plasmid DNA. • Determine the optimal transfection incubation time for each cell type and experiment. Test a range of incubation times (e.g.12-72 hr). The best incubation time is generally 24-48 hr.

	• Precipitate formation during transfection complex formation. • Proper experimental controls were not included. • GeneGlide™ siRNA Transfection reagent was not mixed properly. • Suboptimal GeneGlide™ siRNA Transfection reagent:DNA ratio. • Suboptimal DNA concentration. • Low-quality plasmid DNA. • Endotoxin-contaminated plasmid DNA. • Expressed target gene is toxic to cells. • Cell density not optimal at time of transfection. • Cell morphology has changed.	• Divide the culture at least 18-24 hr before transfection to ensure that the cells are actively dividing and reach optimal cell density at time of transfection. • During complex formation, scale all reagents according to Table 2 including serum-free media, GeneGlide™ siRNA Transfection reagent, plasmid DNA, and siRNA. • Precipitation maybe observed when excess DNA is used during complex formation. This may negatively impact transfection efficiency. To avoid precipitation when using high concentrations of DNA, increase the volume of serum-free medium during complex formation by two-fold. Proper experimental controls were not included. To verify efficient transfection, use GeneGlide™ siRNA Transfection reagent to deliver a positive control such as a green fluorescent protein (GFP) encoding plasmid. • Warm GeneGlide™ siRNA Transfection reagent to room temperature and vortex gently before each use. • Determine the best GeneGlide™ siRNA Transfection reagent: DNA ratio for each cell type. Titrate the GeneGlide™ siRNA Transfection reagent from 2-8 µl per 1 µg DNA. • Determine the DNA concentration accurately. Use plasmid DNA preps that have an A260/280 absorbance ratio of 1.8-2.0. The optimal DNA concentration generally ranges between 1-3 µg/well of a 6-well plate. Start with 2.5 µg/well of a 6-well plate. Consider testing more or less DNA while scaling the amount of GeneGlide™ siRNA Transfection reagent accordingly. • Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. Alternatively, use cesium chloride gradient or anion exchange purified DNA which contains levels of endotoxin that do not harm most cells. Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin.
HIGH CELLULAR TOXICITY	• Transfection complexes and cells not mixed thoroughly after complex addition. • Transfection complexes added to cells cultured in serum-free medium. • Medium change or addition may be necessary. • Knockdown of an essential gene. • Cell density not optimal at time of transfection. • Cell morphology has changed.	• Add transfection complexes drop-wise to the cells. Gently rock the dish back-and-forth and from side-to-side to distribute the complexes evenly. Do not swirl or rotate the dish, as this may cause uneven distribution. • GeneGlide™ siRNA Transfection reagent efficiently transfects cells cultured in serum-free medium; however, toxicity may be higher if serum is not present. If toxicity is a problem, consider adding serum to the culture medium. • If incubating for 48-72 hr, it may be necessary to change the complete medium 24 hr post-transfection. Alternatively, add additional complete medium 4-24 hr post-transfection. • If the siRNA is directed against a gene that is essential to the cell, cytotoxicity may be observed due to knockdown of the target gene. Include a transfection control with non-targeting siRNA to compare the cytotoxic effects of the gene being knocked down. • Determine optimal cell density for each cell type to maximize transfection efficiency. Use this density to ensure reproducibility. For most cell types, ≥80% confluence is recommended at transfection, but use of higher or lower densities may increase cell viability depending on cell type. • Mycoplasma contamination can alter cell morphology and affect transfection efficiency. Check your cells for mycoplasma contamination. Use a fresh frozen stock of cells or use appropriate antibiotics to eliminate mycoplasma. A high or low cell passage number can make cells more sensitive and refractory to transfection. Maintain a similar passage number between experiments to ensure reproducibility.

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