GeneGlide™ DNA Transfection Reagent

(Catalog # M1080-300, -500, -1000; Store at -20°C)

I. Introduction:

BioVision's GeneGlide™ DNA Transfection reagent proprietary lipid-polymer mixture that forms lipopolyplexes with DNA. It is a high-performance, animal-origin free, broad spectrum reagent that provides exceptional transfection of plasmid DNA into mammalian cells. Our transfection reagent can afford high levels of gene expression in typically hard-to-transfect cell types, including primary cells and is suitable for both transient and stable transfections. It is composed of animal-origin free components and is serum compatible, eliminating the need for any culture medium change after transfection. GeneGlide™ DNA Transfection reagent does not effectively deliver siRNA or miRNA. For high efficiency siRNA delivery and knockdown of target gene expression, GeneGlide™ siRNA Transfection reagent is the preferred recommendation.

II. Sample Type:

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

III. Package Contents:

M1080-300	**1 X 300 µl**
M1080-500	**1 X 500 µl**
M1080-1000	**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
- Optional: Selection antibiotic (e.g., G418 or Hygromycin B) for stable transfection

V. Shipment and Storage:

All the reagents are shipped on blue ice. GeneGlide™ DNA Transfection reagent is stored at -20°C. 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.

VII. BEFORE YOU START:

Important Tips for Optimal Plasmid DNA Transfection

Optimize reaction conditions for each cell type to ensure successful transfections. The suggestions below yield high efficiency transfection using BioVision's GeneGlide™ DNA 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.
- **DNA purity.** Use highly purified, sterile, and contaminant-free DNA for transfection. Plasmid DNA preparations that are endotoxin-free and have $A_{260/280}$ 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 Endotoxin Removal Kit to remove endotoxin from your DNA preparation.
- **Ratio of GeneGlide™ DNA Transfection reagent to DNA.** Determine the best DNA Transfection Reagent:DNA ratio for each cell type. Start with 3 µl of GeneGlide™ DNA Transfection reagent per 1 µg of DNA. Vary the concentration of GeneGlide™ DNA Transfection reagent from 1-4 µl per 1 µg DNA to find the optimal ratio. Table 1 provides recommended starting conditions based on cell culture vessel size.
- **Complex formation conditions.** Prepare GeneGlide™ DNA 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, with or without serum. There is no need to perform a medium change to remove the transfection complexes.
- **Presence of antibiotics:** Antibiotics may inhibit transfection complex formation and therefore should be excluded from the complex formation step. Transfection complexes can be added directly 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 cell doubling time.

Table 1. Recommended starting conditions for DNA transfections using GeneGlide™ DNA 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
DNA (1µg/µl stock)	0.1 µl	0.26 µl	0.5 µl	1 µl	2.5 µl	15 µl	19 µl
GeneGlide™ DNA Transfection reagent	0.3 µl	0.78 µl	1.5 µl	3 µl	7.5 µl	45 µl	57 µl

VIII. PLASMID DNA TRANSFECTION PROTOCOL:

The following procedure describes how to perform plasmid DNA transfections using GeneGlide™ DNA Transfection reagent in 6-well plates. The surface areas of other culture vessels are different and transfections must be scaled accordingly. Appropriately increase or decrease the amounts of serum free medium, GeneGlide™ DNA Transfection reagent, DNA and complete culture medium based on the surface area of the cell culture vessel

1. Transient plasmid DNA transfection protocol per well of a 6-well plate:

A. Plate cells

1. Approximately 18-24 hr before transfection, plate cells in 2.5 ml complete growth medium per well in a 6-well plate. Ideally cells should be ≥80% confluent prior to 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 cell cultures overnight.

B. Prepare GeneGlide™ DNA Transfection reagent:DNA complex (Immediately before transfection)

1. Warm GeneGlide™ DNA Transfection reagent to room temperature and vortex gently before using.
2. Place 250 µl of Opti-MEMI Reduced-Serum Medium in a sterile tube.
3. Add 2.5 µg (2.5 µl of a 1 µg/µl stock) plasmid DNA.
4. Pipet gently to mix completely.
5. Add 7.5µl GeneGlide™ DNA Transfection reagent to the diluted DNA mixture.
6. Pipet gently to mix completely.
7. 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™ DNA Transfection reagent:DNA 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™ DNA Transfection reagent:DNA complexes.
3. Incubate for 24-72 hr. It is not necessary to replace the complete growth medium with fresh medium.
4. Harvest cells and assay as required.

Note: For generating stable cell transfectants, passage cells 48-72 hr post-transfection in complete growth medium containing appropriate selection antibiotics, such as G418 or Hygromycin B. Maintain selection for 1-2 weeks to allow for selection of cells that have undergone stable integration of DNA.

IX. Figures and Data:

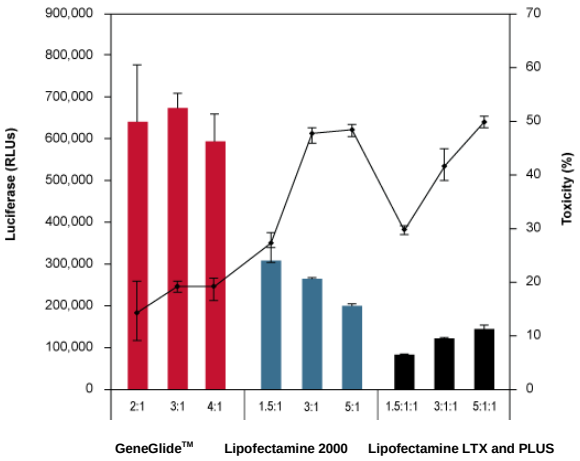

Figure 1. GeneGlide™ Reagent Exhibits Higher Expression and Lower Cellular Toxicity as Compared to Other Transfection Reagents. Human umbilical vein endothelial cells (HUVEC) were transfected with a luciferase expression plasmid using the designated reagents at the reagent-to-DNA ratios indicated beneath each bar. Transfections were performed in 96-well plates using 0.1 µg of plasmid DNA per well. Luciferase expression (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. Error bars represent the standard deviation of triplicate wells.

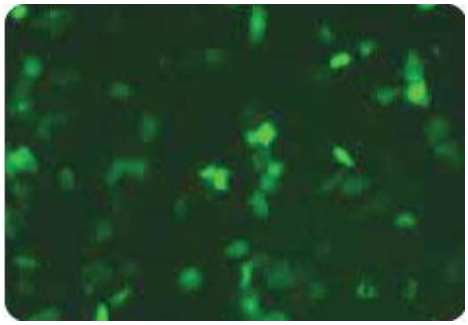

Figure 2. High Performance Plasmid Transfection. Primary Human Small Epithelial Cells (HSAEpic) were transfected using GeneGlide™ and an EGFP expression plasmid (4:1 reagent to DNA ratio). Images were taken 24 hr post-transfection using a Zeiss axiovert inverted fluorescence microscope.

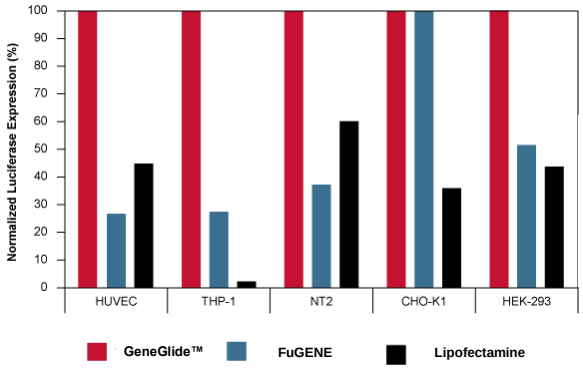

Figure 3. Superior Gene Expression in a Broad Spectrum of Cell Types. The indicated cell types were transfected in 96-well plates with a luciferase expression plasmid (0.1 µg/well) according to industry accepted testing protocols. Reagent to DNA ratios were optimized for each cell type: GeneGlide™ (2:1 or 3:1), FuGENE® HD (Roche, 3.5:1), Lipofectamine® 2000 (Life Technologies, 1.5:1, 3:1 or 5:1). Luciferase activity was measured 24 hr post-transfection. Values were normalized to GeneGlide™ and presented as a percentage of luciferase expression. FuGENE® is a registered trademark of Fugent LLC. Lipofectamine® is a trademark of Life Technologies Corporation.

X. 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™ siRNA Transfection 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

XI. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
LOW PLASMID DNA TRANSFECTION EFFICIENCY	• GeneGlide™ DNA Transfection reagent was not mixed properly. • Suboptimal GeneGlide™ DNA Transfection reagent:DNA ratio. • Suboptimal DNA concentration. • Low-quality plasmid DNA. • Inhibitor present during transfection. • Incorrect vector sequence. • Transfection incubation time. • Cells not actively dividing at the time of transfection. • Precipitate formation during transfection complex formation. • Proper experimental controls were not included.	• Warm GeneGlide™ DNA Transfection reagent to room temperature and vortex gently before each use. • Determine the best GeneGlide™ DNA Transfection reagent:DNA ratio for each cell type. Titrate the reagent from 1-4 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™ DNA Transfection reagent accordingly. • Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. • We recommend removal of endotoxin from your DNA preparation. • 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. • Serum and antibiotics inhibit transfection complex formation. Prepare GeneGlide™ DNA Transfection reagent:DNA 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. • Polyanions such as dextran sulfate or heparin can inhibit transfection. Use culture medium that does not contain these polyanions. If necessary, the transfection medium can be replaced with polyanion containing medium 24 hr post 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. • 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 1 including serum-free medium, GeneGlide™ DNA Transfection reagent and plasmid DNA. • 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. • To verify efficient transfection, use GeneGlide™ DNA Transfection reagent to deliver a positive control such as a luciferase, beta-galactosidase or green fluorescent protein (GFP) encoding plasmid.
HIGH CELLULAR TOXICITY	• Transfection complexes and cells not mixed thoroughly after complex addition. • Transfection complexes added to cells cultured in serum-free medium. • Endotoxin-contaminated plasmid DNA. • Expressed target gene is toxic to cells. • Cell density not optimal at time of transfection. • Cell morphology has changed.	• Add GeneGlide™ DNA Transfection reagent:DNA complexes drop-wise to different areas of the wells containing plated 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. • Allow GeneGlide™ DNA Transfection reagent:DNA complexes to form in serum-free medium, then add these complexes to cells cultured in complete growth medium. The presence of serum in the growth medium improves transfection efficiency and reduces cytotoxicity. No culture medium change is required after the addition of transfection complexes to cells. • Use highly purified, sterile, endotoxin and contaminant-free DNA for transfection. • We recommend Endotoxin Removal Kit for removal of any traces of endotoxin from your DNA preparation. Alternatively, use cesium chloride gradient or anion exchange purified DNA that do not harm most cells. • Do not use DNA prepared using miniprep kits as it might contain high levels of endotoxin. • Compare toxicity levels against a cells alone control and cells transfected with an empty vector to assess the cytotoxic effects of the target protein being expressed. If lower levels of target gene expression are desired in your transfection experiments, consider reducing the amount of target plasmid. Maintain the optimal GeneGlide™ DNA Transfection reagent:DNA ratio by using carrier DNA such as an empty cloning vector. • Determine the best cell density for each cell type to maximize transfection efficiency. Use this cell density in subsequent experiments 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.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com