GeneJammer Transfection Reagent

INSTRUCTION MANUAL

Catalog #204130, #204131, and #204132

Revision A.02

For In Vitro Use Only

204130-12

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GeneJammer Transfection Reagent

Contents

GeneJammer Transfection Reagent

MATERIALS PROVIDED

Materials provided	Quantity[a]		
	Catalog #204130	Catalog #204131	Catalog #204132
GeneJammer transfection reagent	1.0 ml	4 × 1.0 ml	0.3 ml

[a]1.0 ml of GeneJammer transfection reagent provides for over 300 transfections when using 35-mm tissue culture dishes or 6-well culture plates.

Note *A visible precipitate may be seen in the GeneJammer transfection reagent solution. Incubate the vials at room temperature to resolubilize the precipitate before use.*

STORAGE CONDITIONS

GeneJammer transfection reagent: 4°C

ADDITIONAL MATERIALS REQUIRED

Media for preparing transfection mixture (DMEM, RPMI or other cell culture media **without serum and antibiotics**)
Media for cell growth (DMEM, RPMI, or other cell culture media, including serum, growth factors, and antibiotics if appropriate)
1× PBS, sterile
5-ml Falcon® polystyrene round bottom tubes (BD Biosciences catalog #352054)

Revision A.02

INTRODUCTION

Gene transfection into eukaryotic cells is a fundamental tool for analysis of gene function and for production of recombinant gene products. The GeneJammer Transfection Reagent is a proprietary formulation of polyamine and other components in 80% ethanol; this transfection reagent is effective in both serum-containing medium and serum-free medium, offering high efficiencies with minimal cytotoxicity. Easy to use, GeneJammer transfection reagent is highly stable and arrives as a single vial reagent with no reconstitution required.

PREPROTOCOL CONSIDERATIONS

The protocol provided below will allow effective transfections for most cell types, however, optimal transfection conditions may vary. The following parameters should be established for each cell line and plasmid used.

DNA Quality and Concentration

The use of highly purified DNA is critical for successful transfection. Suitable quality DNA is obtained using StrataPrep plasmid miniprep kit, or by cesium chloride purification. The optimal concentration for transfection generally falls within the range of 1–2 μg per 35-mm dish, with 1 μg as the recommended starting point for optimization. The plasmid DNA solution should be in sterile TE or water at a concentration of 0.02–20 μg/μl.

Cell Density

While we recommend a cell density of 50–80% confluence for most cell types, the optimal cell density should be determined specifically for each cell type.

Cell Growth in Serum-free media

The protocol outlined in this manual uses serum-containing medium during the transfection procedure. If you choose to perform the transfection in serum-free medium, replace the medium with serum-containing medium 3–8 hours after transfection.

Reagent to DNA Ratio

The optimal ratio of GeneJammer reagent (µl) to DNA (µg) should allow the highest transfection efficiencies with the lowest level of toxicity. For most cell lines, a reagent to DNA ratio of 3:2, 3:1, or 6:1 is best. These ratios are a good starting point for optimization experiments with your specific cell line and DNA. See the figure below for a suggested layout of a 6-well plate that tests all three of these ratios. To optimize further, keep the quantity of DNA constant and add increasing volumes of transfection reagent. Excess DNA may inhibit transfection efficiency, so do not exceed a reagent:DNA ratio of 3:2.

(A) Cell Control No reagent No DNA	**(B) Reagent Control** 6 µl reagent No DNA	**(C) DNA Control** No reagent 1 µg DNA
(D) 3:1 ratio 3 µl reagent 1 µg DNA	**(E) 3:2 ratio** 3 µl reagent 2 µg DNA	**(F) 6:1 ratio** 6 µl reagent 1 µg DNA

GENEJAMMER TRANSFECTION REAGENT VOLUME GUIDELINES

The following procedure for the generation of transfectants uses 35-mm tissue culture dishes or 6-well culture plates. The optimal ratio of GeneJammer transfection reagent volume (µl) to µg of DNA must be determined for each plasmid and cell line, but ratios of 3:2, 3:1 and 6:1 are recommended as starting points for optimization. Table I lists volume guidelines for generating reagent:DNA mixtures of a 3:1 ratio for commonly used tissue culture dishes. To prepare mixtures of a 3:2 ratio, double the quantity of DNA listed in the table. To prepare mixtures of a 6:1 ratio, double the volume of GeneJammer reagent listed in the table. The volume of the transfection mixture may be scaled up by increasing the components proportionally to accommodate several transfections.

TABLE I

GeneJammer transfection reagent use for different cell culture volumes [a]

Tissue culture dish format	Diameter of the well (mm)	Volume of GeneJammer transfection reagent (µl/well)	Amount of DNA per well (µg)	Final volume of transfection mixture (µl/well)	Typical volume of medium per well (ml)
96-well	6.4	0.15	0.05	5	0.1–0.2
48-well	10	0.3	0.1	10	0.2–0.5
24-well	15	0.6	0.2	20	0.5–2.0
12-well	22	1.5	0.5	50	0.5–1.0
6-well	35	3.0	1.0	100	2.0
35-mm	35	3.0	1.0	100	2.0
60-mm	60	6.0	2.0	200	4.0–6.0
100-mm	100	18.0	6.0	600	10.0

[a]These are estimated starting parameters and transfection conditions may require optimization.

TRANSFECTING CELLS

Preparing the Cells for Transfection (Adherent cells)

1. Twenty-four hours before transfection, inoculate a 6-well tissue culture plate (or 35-mm culture dish) with $1–3 \times 10^5$ exponentially growing cells per well.

2. Grow the cells overnight in an appropriate volume of their growth medium. The cells should be 50–80% confluent at the time of transfection.

Preparing the Cells for Transfection (Suspension cells)

1. Grow cells in growth medium to a concentration of 5×10^4 to 1×10^6 cells per ml in a 6-well tissue culture plate or 35-mm culture dish.

Notes *Determine the optimal concentration for your cell type and DNA. Cells should be in log-growth phase.*

Preparing the Transfection Mixture

Perform this procedure immediately prior to transfection.

Notes *A visible precipitate may be seen in the GeneJammer transfection reagent solution. Incubate the vials at room temperature to resolubilize the precipitate before use.*

The optimal ratio of GeneJammer transfection reagent to DNA must be determined for each plasmid and cell line. We recommend reagent to DNA ratios of 3:2, 3:1 and 6:1 as a starting point for optimization. Refer to Table I for guidelines.

1. Transfer 97 µl of sterile, room temperature, **serum-free**, antibiotic-free DMEM or RPMI (or medium of choice) to a polystyrene tube.

   Note *The total volume of medium plus transfection reagent should be 100 µl. If using more than 3 µl of transfection reagent in the following step, adjust the volume of medium at this step accordingly.*

2. Add 3 µl GeneJammer transfection reagent by pipetting directly into serum-free medium. **The presence of serum at this stage is inhibitory**. To mix, stir gently with the pipet tip. Do not vortex.

Note *Avoid allowing the undiluted reagent to contact the plastic tube directly. This greatly reduces the efficacy of the reagent.*

3. Incubate at room temperature for 5 minutes.

4. Add the DNA to the diluted GeneJammer transfection reagent and mix gently. For stable transfections, prepare a negative control.

   Note *The volume of DNA added should be between 0.5–50 µl.*

5. Incubate for 15–45 minutes at room temperature.

Adding the Transfection Mixture

1. Remove the prepared culture of cells from the incubator. It is not necessary to remove the growth medium.

2. Add the transfection mixture dropwise to the tissue culture dish. Gently rock the plate back and forth to distribute the transfection mixture evenly. Incubate the plate in standard growth conditions (i.e., 37°C and 5% CO_2 in a humidified incubator).

   Note *For most commonly used laboratory cell lines, the cells can continue to incubate in the presence of the transfection reagent until the time of the gene expression assay.*

3. For transient transfection, continue to incubate the transfected cells for 24–72 hours, depending on the cell type, reporter system, and promoter activity. For stable transfection, proceed to *Performing a Stable Transfection*.

Performing a Stable Transfection (Adherent Cells)

1. Perform transient transfection as described above. After 24–72 hours in growth medium (or until cells are confluent), split the cells to the desired ratio (at least 1:5) into growth medium containing the selection antibiotics at a concentration appropriate to the cell line.

2. Replace the medium and apply fresh selection antibiotics every 4–7 days (approximately two times per week).

3. Stable colonies form within 1–2 weeks. Cells from the negative control DNA dish die off.

Performing a Stable Transfection (Suspension Cells)

1. Perform transient transfection as described above. After 24–72 hours in growth medium, split the cells to the desired ratio (at least 1:5) into growth medium containing selection antibiotics at a concentration appropriate to the cell line.

2. Separate viable from non-viable cells with the method of choice. Replace the medium and apply fresh selection antibiotics every 4–7 days (approximately two times per week) until the desired quantity of viable cells are present.

TROUBLESHOOTING

Observation	Suggestion
The transfection efficiency is low	Determine the optimal ratio of GeneJammer transfection reagent to DNA for each cell type. We recommend ratios of 3:2, 3:1 and 6:1 as the starting points for optimization.
	Ensure that the GeneJammer transfection reagent does not contact the side of the polystyrene tube when the transfection mixture is prepared.
	Confirm that the medium used for the transfection mixture is serum-free.
	Ensure the cells are 50–80% confluent at the time of transfection.
	Ensure the plasmid DNA has an $OD_{260/280}$ ratio of ~ 1.8–2.0 and is endotoxin free.
	Execute a positive control for the transfection assay.
	Ensure the promoter element is expressed in the cell type used.
The incidence of cell toxicity is high	Execute a dose-response curve varying the amount of transfection reagent while maintaining the amount of DNA to determine if too much reagent is used; alternatively, execute a dose-response curve varying the amount of DNA while maintaining the amount of transfection reagent to determine if too much DNA is used.
	Increase the density of the cells used.
	Some cell lines may require at least 48 hours for cells to express resistance genes before adding selection antibiotics.
	For cell lines that exhibit sensitivity to the GeneJammer transfection reagent during the initial incubation period, complete removal of the reagent 3–8 hours after transfection may be desirable.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

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GeneJammer Transfection Reagent

Catalog #204130, 204131, and 204132

QUICK-REFERENCE PROTOCOL

Prepare Cells (Adherent cells)
Seed 1–3 × 10^5 cells per 35-mm culture dish or per well of a 6-well plate and incubate until 50–80% confluent

Prepare Cells (Suspension cells)
Grow cells to concentration of 5 × 10^4 to 1 × 10^6 cells per ml in a 35-mm culture dish or 6-well plate in growth medium

Prepare Transfection Mixture (for 3:1 reagent to DNA ratio)

- Pipet 97 µl serum-free medium (room temperature) into polystyrene tube
- Pipet 3 µl reagent **into** medium, do not touch plastic
- Incubate 5 minutes at room temperature
- Add 1 µg DNA and incubate for 15–45 minutes at room temperature (prepare a DNA negative control if performing stable transfection)

Transfect Cells

- Add the transfection mixture dropwise to the cells; rock the dish or plate gently to distribute the transfection mixture
- For transient transfection, incubate cells for 24–72 hours in standard growth conditions before performing gene expression assay

Perform Stable Transfection (optional)

- After 24–72 hours, split the cells to the desired ratio (at least 1:5) into growth medium containing selection antibiotics at appropriate concentration

Adherent cells

- Replace the medium and apply fresh selection antibiotics every 4–7 days
- Stable Colonies form within 1–2 weeks; cells from the negative control plate die off

Suspension cells

- Separate viable from nonviable cells with the method of choice
- Replace the medium and add fresh antibiotics every 4–7 days until the desired quantity of viable cells are present