SatisFection Transfection Reagent

Instruction Manual

Catalog #204121, #204122, and #204123

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

204121-12

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SatisFection Transfection Reagent

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SatisFection Transfection Reagent

MATERIALS PROVIDED

Materials provided	Quantity[a]		
	Catalog #204121	Catalog #204122	Catalog #204123
SatisFection Transfection Reagent	0.3 ml	0.75 ml	2 × 0.75 ml

[a] The reagent has been optimized for 625–1250 transfections per 0.75 ml transfection reagent using 24-well tissue culture dishes.

STORAGE CONDITIONS

SatisFection Transfection Reagent: 4°C

ADDITIONAL MATERIALS REQUIRED

Media for preparing transfection mixture (Opti-MEM, DMEM, RPMI or other cell culture media **without serum and antibiotics**)

Media for cell growth (Opti-MEM, DMEM, RPMI, or other cell culture media, including serum, growth factors, and antibiotics if appropriate)

Revision B

INTRODUCTION

Gene transfection into eukaryotic cells is a fundamental tool for analysis of gene function and for production of recombinant gene products. SatisFection Transfection Reagent gives optimal efficiencies and protein expression levels in common cell lines, while significantly improving efficiencies in many hard-to-transfect and primary cells. Maximum gene expression and protein production is observed 36–72 hours post-transfection.

The SatisFection reagent is a novel cationic polymer suitable for both transient and stable transfections in the presence or absence of serum. This polymer functions by binding and condensing DNA into a polyplex that enters the cell via endocytosis. Properties of the polyplex facilitate both endosomal rupture and protection of the bound DNA from lysosomal degradation. Continued exposure to cellular pH ultimately reduces the polymer into a non-toxic monomer. The monomer is then easily excreted from the cellular compartment, resulting in lowest the levels of cytotoxicity and increased cell viability. As a result, the SatisFection reagent is ideal for experiments that require large, healthy populations of positively transfected cells for downstream applications, e.g., signal transduction pathway analysis.

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 0.4–1.0 µg DNA per well of a 24-well plate, with 0.6 µg as the recommended starting point for optimization.

Cell Density

While we recommend a cell density of 60–80% confluence after 18–24 hours of incubation at the time of transfection for most cell types, the optimal cell density should be determined specifically for each cell type.

Reagent to DNA Ratio

The optimal reagent to DNA ratio should allow the highest transfection efficiencies with the lowest level of toxicity. This is usually achieved within the range of 1–2 µl reagent per µg DNA, with 1.5 µl reagent per µg DNA the recommended starting point for optimization.

Transfection Incubation Time

The optimal time that the transfection reagent is in contact with the cells should be determined by testing a range of incubation times from 36–72 hours.

SATISFECTION TRANSFECTION REAGENT VOLUME GUIDELINES

The following procedure for the generation of transfectants uses a 24-well tissue culture plate. The optimal ratio of SatisFection transfection reagent to DNA must be determined for each plasmid and cell line, however 1.5 µ1 reagent per 1 µg DNA is the recommended starting point for optimization. Reagent volumes for cells not cultured in 24-well tissue culture plates are given in Table I. When preparing the transfection mixture, the volume of the transfection mixture may be scaled up by increasing the components proportionally to accommodate several transfections.

TABLE I

SatisFection Transfection Reagent use for Various Cell Culture Dish Formats [a]

Tissue culture dish format	Diameter of the well (mm)	Recommended number of adherent cells ($\times 10^5$)	DNA solution per well		Transfection reagent per well		Total volume of transfection mixture plus medium (µl)
			Amount of DNA (µg)	Final volume of DNA solution diluted with serum-free medium (µl)	Volume of transfection reagent stock solution (µl) [1.5:1 ratio (µl reagent: µg DNA)]	Final volume of transfection reagent solution diluted with serum-free medium (µl)	
96-well	6.4	0.04–0.1	0.15	7.5	0.225	7.5	15 µl
48-well	10	0.1–0.3	0.3	15	0.45	15	30 µl
24-well	15	0.27–0.67	0.6	30	0.9	30	60 µl
12-well	22	0.6–1.5	1	50	1.5	50	100 µl
6-well	35	1.5–3.7	2	100	3	100	200 µl
35-mm	35	1.5–3.7	2	100	3	100	200 µl
60-mm	60	4.0–11.0	6	300	9	300	600 µl
100-mm	100	12.0–31.0	16	800	24	800	1600 µl

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

TRANSFECTING ADHERENT CELLS

Note *Reagent volumes for cells not cultured in 24-well tissue culture plates are given in Table I. When preparing the transfection mixture, the volume of the transfection mixture may be scaled up by increasing the components proportionally to accommodate several transfections.*

Preparing the Cells for Transfection

1. Eighteen to twenty-four hours before transfection, inoculate a 24-well tissue culture plate with $2.7–6.7 \times 10^4$ exponentially growing cells per well. The cells should be 60–80% confluent at the time of transfection.

2. Grow the cells overnight according to standard cell culture conditions in an appropriate volume of their growth medium (~500 µl medium).

Preparing the Transfection Mixture

Perform this procedure immediately prior to transfection.

Notes *The amounts given in the following protocol are for one transfection, or well, and can be adjusted for multiple samples by multiplying each amount by the number of transfections.*

The optimal ratio of SatisFection transfection reagent to DNA must be determined for each plasmid and cell line. We recommend 1.5 µl SatisFection transfection reagent per 1 µg DNA as a starting point for optimization.

For stable transfections, prepare a negative control.

1. Transfer 30 µl of sterile, room temperature, serum-free, antibiotic-free DMEM (or medium of choice) to a microcentrifuge tube.

2. Add the SatisFection transfection reagent (0.9 µl) 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.

3. In a separate microcentrifuge tube, add 30 µl of sterile, room temperature, serum-free, antibiotic-free DMEM (or medium of choice).

4. Add the experimental DNA (0.6 µg) to the separate tube of serum-free medium. To mix, vortex gently.

5. After diluting the transfection reagent and DNA separately, add the diluted transfection reagent dropwise to the diluted DNA while gently vortexing or mixing with the pipet tip. The total transfection mixture volume is ~60 µl.

6. Incubate the transfection mixture for 15 minutes at room temperature.

Adding the Transfection Mixture

1. Add the transfection mixture dropwise to the well. Ensure that the mixture is added directly into the medium, rather than the sides of the well. Do not disrupt the cells. Gently rock the dish back and forth to distribute the transfection mixture evenly.

   Note *A media change is not required prior to adding the transfection mixture to the cells. The medium may contain serum or be serum-free, and may contain antibiotics. In general, We have found that the SatisFection transfection reagent yields improved transfection efficiency when transfection of adherent cells is performed in serum-containing medium.*

2. For transient transfection, incubate for 36–72 hours using standard growth conditions (i.e., 37°C and 5% CO_2 in a humidified incubator), depending on the cell type, reporter system, and promoter activity. For stable transfection, proceed to *Performing a Stable Transfection*.

   Note *If cells display sensitivity to the transfection mixture, the medium may need to be changed 4–6 hours after the addition of the transfection mixture.*

Performing a Stable Transfection

1. Perform transient transfection as described above. After 36–72 hours in growth medium, split the cells to the desired ratio (at least 1:5) into growth medium.

2. After 24 hours, apply selection antibiotics dropwise to the tissue culture dish, swirling the dish between drops, at a concentration appropriate to the cell line.

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

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

TROUBLESHOOTING

Observation	Suggestion
The transfection efficiency is low	The strong affinity of SatisFection for plasmid DNA requires longer incubation times, allow your cells to grow between 36–72 hours.
	Determine the optimal ratio of SatisFection transfection reagent:DNA for each cell type.
	Optimize the amount of the plasmid DNA used for each cell type.
	Confirm that the medium used for the transfection mixture is serum-free.
	Ensure the cells are 60–80% 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.
	Do not add antibiotics to the medium during the transfection and allow at least 24 hours for cells to express resistance genes before adding selection antibiotics.
	For cell lines that exhibit sensitivity to the SatisFection transfection reagent during the initial incubation period, complete removal of the reagent prior to the 36–72 hour incubation period may be desirable.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.

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QUICK-REFERENCE PROTOCOL–ADHERENT CELLS

Seed cells to 2.7–6.7 $\times$ 10^4 cells per well of a 24-well plate and incubate 18–24 hours so that cells are at 60–80% confluency at time of transfection

Prepare transfection mixture

- Pipet 30 µl antibiotic-free, serum-free medium (room temperature) into microcentrifuge tube. Pipet reagent (0.9 µl) **into** medium, do not touch plastic.
- Pipet 30 µl antibiotic-free, serum-free medium (room temperature) into separate microcentrifuge tube. Add DNA (0.6 µg) to medium.
- Add the diluted transfection reagent dropwise to the diluted DNA while gently vortexing or mixing with the pipet tip.
- Incubate transfection mixture 15 minutes at room temperature.

Add the transfection mixture to the cells

- Add transfection mixture dropwise and incubate for 36–72 hours under standard growth conditions.

Perform stable transfection (optional)

- 36–72 hours after the transfection (above), split cells (1:5 suggested).
- After 24 hours, apply selection antibiotics.
- Continue selection, replacing medium every 4–7 days until colonies form.