Quantum Prep® Cytofectene™ Transfection Reagent Kit

Catalog Numbers
170-3250, 170-3251, 170-3252

Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA 94547

4006154 Rev B

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Section 1
Introduction to the Quantum Prep Cytofectene Transfection Reagent Kit

1.1 Overview

The Cytofectene transfection reagent is a mixture of two proprietary compounds, a cationic lipid with a charge of +4 under physiological conditions and a neutral lipid. These compounds have been optimized for intracellular delivery of plasmids to cultured mammalian cells at high efficiency in the presence of serum. For most cell lines, high levels of expression can be obtained using concentrations of Cytofectene suggested in this manual for given sizes of plates/wells. However, for best results it is important to determine the optimum concentration of Cytofectene for each cell line of interest.

1.2 Contents

The Cytofectene transfection reagent contains either 0.2 ml (catalog number 170-3252), 1 ml (catalog number 170-3250), or 5 x 1 ml (catalog number 170-3251) of a proprietary lipid formulation that is ready to use for delivery of plasmids to cultured cells. One milliliter is generally sufficient for 125–200 cell transfections in 35 mm plates.

1.3 Storage and Stability

The Cytofectene transfection kit is shipped on wet ice. Store at 4 ºC upon receipt. **Do not store below 0 ºC**. The Cytofectene transfection kit is stable for 1 year from date of purchase when stored at 4 ºC. The reagent is stable for short periods of time at room temperature and need not be kept on ice while setting up experiments.

Section 2
Protocol

2.1 Recommendations for Best Results

The following protocol describes transfection of adherent cells in 6 well plates or 35 mm dishes in a final transfection media volume of 1 milliliter. Table 1 gives suggested ranges of Cytofectene, plasmid DNA, and total amounts of transfection media volumes for the remaining commonly used sizes of cell culture dishes. Section 3 contains protocols for optimizing the concentration of Cytofectene and the amount of plasmid in order to maximize transfection efficiency for a cell line of interest, as well as a more detailed listing of starting conditions for transfection of cells in various-sized tissue culture dishes.

2.2 Helpful Hints

- Use sterile polystyrene plasticware, *e.g.*, 12 x 75 mm tubes or multi-well trays, to prepare the 10x plasmid solutions. Polystyrene is recommended because cationic lipid-plasmid complexes may bind to polypropylene.
- When beginning or first optimizing experiments with Cytofectene, use the same percentage of serum that cells have been adapted to in culture. Standard concentrations of the antibiotics penicillin (100 U/ml) and streptomycin (100 µg/ml) may be included in the media.
- The type of media has a role in transfection efficiency. Maximum transfection efficiency for some cell lines is found in media formulated for use in reduced serum conditions.
- The percentage of serum in the transfection medium has a role in transfection efficiency. Compare gene expression following transfection at concentrations of serum varying from 2-10% to identify the optimum serum concentration.
- It is usually not necessary to remove the DNA/Cytofectene solution from the cells. For some cell lines, adding an equal volume of standard growth media to the cells 4–6 hours following addition of the DNA/Cytofectene solution results in increased gene expression and cell viability. If toxicity is observed, aspirate the transfection media after 4–6 hours and replace with fresh media.
- For stable expression of the transfected plasmid sequence, remove transfection media 24 hours after transfection and trypsinize the cells. Transfer the cells to a fresh plate with growth media containing no selective agent. The following day,

replace the media with media plus the selective agent to be employed. Continue incubating for 1–2 weeks to allow growth of the cells expressing the transfected gene.

2.3 Protocol for Transfection of Adherent Cells (6 well/35 mm plates)

1. The day before transfection, inoculate an appropriate number of cells into 6 well plates or 35 mm dishes such that they will be 50–80% confluent the following day. For most cells, plating 2–8 x 10^5 cells in 2.0 ml of media should be appropriate. Incubate the cells at 37 °C in a 5% CO_2 incubator overnight.
2. Prepare a 10x solution of plasmid DNA in serum-free media, *i.e.*, dilute 0.5–2 µg of plasmid to a total volume of 100 µl with serum-free media in a sterile 12 x 75 mm polystyrene tube for each plate of cells to be transfected.
3. Add the required amount of Cytofectene, *i.e.*, 5-8 µl, to the 10x plasmid DNA solution. Mix gently by tapping or pipetting and incubate 10–20 minutes.
4. Add 900 µl of serum-containing media to the combined 10x Cytofectene/plasmid solutions for a final volume of 1 ml. Use the same percentage of serum to which cells have been adapted in culture. Avoid vortexing or centrifuging.
5. Remove the media from the cells to be transfected and replace it with the 1.0 ml of the serum-containing media with the Cytofectene/plasmid complexes. Incubate the cells at 37 °C in a CO_2 incubator.

6. For transient expression, assay reporter gene activity 24–48 hours after the start of transfection.

Table 1. Suggested Reagent Quantities for Different Sizes of Plates/Wells

Plate Size	Plasmid DNA	10x Volume Plasmid+ Serum-Free Media	Cytofectene	Volume Serum-containing Media	Total Volume Applied to Plate/Well
96 well	50–200 ng	10 µl	0.5–0.8 µl	90 µl	100 µl
48 well	100–400 ng	20 µl	1.0–1.6 µl	180 µl	200 µl
24 well	200–800 ng	40 µl	2.0–3.2 µl	360 µl	400 µl
12 well	250–1000 ng	50 µl	2.5–4.0 µl	450 µl	500 µl
6 well/35 mm	0.5–2 µg	100 µl	5.0–8.0 µl	900 µl	1 ml
60 mm	1.0–4.0 µg	200 µl	10–16 µl	1.8 ml	2 ml
100 mm	3.5–14 µg	700 µl	35–56 µl	6.3 ml	7 ml

2.4 Protocol for Transfection of Suspension Cells (6 well/35 mm plates)

The following protocol is for transfection of 0.5–2 x 10^6 suspension cells in 6 well plates or 35 mm dishes. Table 3 (Section 3.4) gives additional suggestions for larger or smaller plates/wells, adjusting all amounts in proportion to changes in the volume of media used.

The day before transfection, dilute the cells such that they will be in log phase growth the following day. For most cells, inoculation of 0.6–2.5 x 10^5 cells per ml of media should be appropriate. Incubate the cells at 37 °C in a CO_2 incubator overnight (16–24 hours).

1. Pellet the cells in a tabletop centrifuge at 300–500 x g for 7–8 minutes. Resuspend the cell pellet at 0.6–2.5 x 10^6 cells/ml of growth media. Use the same percentage of serum in the media to which the cells have been adapted in culture. Inoculate 0.9 ml of cells into 35 mm dishes.
2. Prepare a 10x solution of plasmid DNA in serum-free media, *i.e.*, dilute 0.5–2 µg plasmid to 100 µl with serum-free media for each plate of cells to be transfected in a sterile polystyrene tube.
3. Add the required amount of Cytofectene, *i.e.*, 5–8 µl, to the 10x plasmid DNA solution. Mix gently by tapping or pipetting and incubate 10–20 minutes.
4. Add the 100 µl of the combined 10x Cytofectene/plasmid mixture to the cell suspension; rock the plates gently to ensure adequate mixing of the solutions.

5. Incubate the cells at 37 °C in a CO_2 incubator.
6. For transient expression, assay reporter gene activity 24–48 hours after the start of transfection.

Section 3 Maximizing Transfection Efficiency

3.1 Optimization Issues

Determining the optimum conditions for transfection efficiency is essential for maximal gene expression and to minimize toxicity to the cells. Some important parameters are the concentration of transfection reagent, the amount of plasmid being delivered, and the concentration of serum in the transfection media. Cell densities can also affect transfection efficiency and may also result in differences in cell viability following transfection.

The two most important parameters to optimize are the concentration of Cytofectene and the amount of plasmid, in that order. The optimal concentration of Cytofectene for adherent cells is usually in the range of 5–8 µl/ml after combination with plasmid DNA and transfection media. In general, gene expression increases with increasing concentrations of Cytofectene, plateaus, and then decreases. This decrease in gene expression correlates with reduced cell viability. Gene expression also increases with increasing amounts of plasmid added to the cells, then plateaus or even decreases. The concentration of Cytofectene and the amount of plasmid required for maximal expression can vary from

one cell line to another. These should be assessed in order to determine the optimum transfection conditions if absolutely maximal expression is required for the experimental plan.

Section 3.2 includes a protocol for determining the optimum Cytofectene concentration for any cell line. Table 2 shows transfection data with some commonly used mammalian cells along with recommended concentrations of Cytofectene to use as a starting point for optimizing transfection.

Table 2. Optimum Concentrations of Cytofectene for Luciferase and GFP Expression In Selected Cell Lines

Cell line	Cytofectene (µl/ml)	Luciferase expression[1] (RLU/10^{5}cells)	GFP expression[2] (% GFP+ cells)	Viability (%)
CHO	5	2–4 x 10^6	44	94
COS7	12	8–12 x 10^6	87	87
3T3	6	1–7 x 10^5	59	96
BHK	5	9–17 x 10^6	46	98
HeLa	5	1–3 x 10^4	40	83
293	8	8–9 x 10^6	76	86
HepG2	7	5–6 x 10^5	81	72
A549	8	0.3–2 x 10^5	62	ND
PC12	5	3–4 x 10^4	80	90

(1) 1 µg of pCMViLuc+ was delivered to cells using the indicated volume of Cytofectene as described in Section 2.3; 24 hours later cells were lysed and assayed for luciferase activity.

(2) 1 µg of pGreenLantern (Life Technologies, Inc.) was delivered to cells using the indicated volume of Cytofectene as described in Section 2.3; 24 hours later, cells were trypsinized, resuspended in PBS containing 1 g/L glucose and 1 µg/ml propidium iodide, then assayed for viability and GFP expression in a Bio-Rad BRYTE flow cytometer.

Section 3.3 describes a method for assessing the amount of plasmid that results in maximal gene expression. Adding additional transfection reagent does not result in higher gene expression at high DNA concentrations. The media in which the cells are transfected, the percentage of serum in the transfection media, and supplementing (diluting) the transfection media 4–6 hours following gene delivery are additional factors that can affect the level of gene expression.

Optimizing transfection conditions is most readily accomplished using a plasmid containing a reporter gene that may be easily assayed, such as chloramphenicol acetyltransferase (CAT), luciferase (luc), β-galactosidase (β-gal), human growth hormone (hGH), secreted alkaline phosphatase (SEAP), and green fluorescent protein (GFP). 24–48 hours following delivery of the reporter gene, expression may be assayed either in the media (hGH, SEAP), in cell extracts (luc, β-gal, CAT) or in live (GFP) or fixed (GFP, β-gal) cells.

3.2 Optimizing the Amount of Cytofectene (Adherent Cell Cultures)

This optimization protocol utilizes 6 well plates or 35 mm diameter dishes. See Section 3.4 for guidelines on optimizing in other sizes of plates/wells. The samples are assayed in duplicate with 1 µg of plasmid per dish and with the Cytofectene concentration ranging from 0–12 µg per plate as indicated in the table on the opposite page. Additionally, the two remaining wells may be used as a no plasmid, no Cytofectene control.

1. Inoculate cells to be tested into each well of two 6 well plates. Grow the cells until they are 50–80% confluent.
2. Prepare a master 10x plasmid DNA solution containing 1 µg of plasmid per 100 µl of media, *i.e.*, combine 12 µg of plasmid and serum-free media in a final volume of 1.2 ml serum-free media.

   Note: The concentration of plasmid stock should be at least 0.2 µg/µl (in TE or water).

3. Dispense 200 µl of the 10x plasmid DNA solution into each of five 12 x 75 mm polystyrene tubes or into each of five wells of a 12 well plate.
4. Add four different volumes of Cytofectene as indicated in the table below to each 10x plasmid DNA solution; mix, and incubate for 10–20 minutes.

Tube	Cytofectene (µl)	Serum-free Media (µl)	Final Cytofectene Conc. (µg/ml)
1	0	200	0
2	4	196	2
3	8	192	4
4	16	184	8
5	24	176	12

5. Add 1.8 ml of growth media (containing the same percentage of serum that cells have been adapted to in culture) to each 200 µl of 10x Cytofectene/plasmid solution.
6. Remove the media from each plate of cells to be transfected; add 1 ml of each of the diluted Cytofectene/plasmid solutions to cells in duplicate. Repeat with each of the Cytofectene concentrations and with the controls. Incubate the cells at 37 °C in a CO_2 incubator.
7. Assay expression of the reporter gene 24–48 hours post-transfection to identify the optimal Cytofectene concentration.

3.3 Optimizing Amount of Plasmid (Adherent Cell Cultures)

It is recommended that the Cytofectene concentration be optimized prior to optimizing plasmid concentration (see Section 3.2). Most cationic lipid transfection agents are functional only within a narrow concentration range based on the charge ratio of the lipid/DNA complex. However, the concentration of Cytofectene optimized for one plasmid concentration is optimal over a broad range of plasmid concentrations. This optimization protocol utilizes a 6 well plate (35 mm diameter) format. See Section 3.4 for guidelines on optimizing in other sizes of plates/wells.

1. Inoculate cells to be tested into each well of two 6 well plates. Incubate the cells in a 37 °C incubator with CO_2 until they are 50–80% confluent.
2. Prepare five concentrations of 10x plasmid DNA solutions in sterile 12 x 75 mm polystyrene tubes or in five of the wells of a 12 well plate as indicated in the table below. The 200 µl volume of DNA + media is sufficient for the transfection of duplicate samples at each concentration.

Tube	Plasmid	Serum-free Media (µl)	Final DNA conc. (µg/ml)
1	2 µl @ 0.1 µg/µl	198	0.1
2	5 µl @ 0.1 µg/µl	195	0.25
3	10 µl @ 0.1 µg/µl	190	0.5
4	2 µl @1.0 µg/µl	198	1
5	4 µl @ 1.0 µg/µl	196	2

3. To each 10x plasmid DNA solution, add twice the optimum volume of Cytofectene required for transfecting the cells on one 35 mm plate, mix, and incubate 10–20 minutes.
4. To each combined 10x DNA/Cytofectene solution, add 1.8 ml of growth media containing the same percentage of serum to which cells have been adapted in culture.
5. Remove the media from each plate of cells to be transfected; add 1 ml of each of the diluted Cytofectene/plasmid solutions to cells in duplicate. Repeat with each of the Cytofectene concentrations and with the controls. Incubate the cells at 37 °C in a CO_2 incubator.
6. Assay expression of the reporter gene 24–48 hours post-transfection to identify the optimal plasmid amount.

3.4 Transfection of Cells in Different-Sized Dishes

Table 3. Suggested cell densities, Cytofectene/plasmid amounts, and media volumes for various-sized tissue culture plates.

		Adherent Step 1	Adherent Step 1	Suspension Step 1	Suspension Step 1	Step 2	Step 2	Step 3	Step 4	Step 5
Plate Size	**Area (cm^2)**	**Cell Density of Inoculum ($x10^5$/plate)**	**Media Volume per plate**	**Cell density ($x10^5$/ml)**	**Cell Volume of Inoculum per plate**	**Plasmid DNA**	**10xVolume Plasmid +Serum-Free Media**	**Cytofectene (µl/plate)**	**Volume Serum containing media (for adherent cells)**	**Total Volume Applied to Plate/Well**
96 well	0.32	0.07–0.28	100 µl	0.6–2.5	90 µl	50–200 ng	10 µl	0.5–0.8	90 µl	100 µl
48 well	1.0	0.2–0.8	300 µl	0.6–2.5	180 µl	100–400 ng	20 µl	1.0–1.6	180 µl	200 µl
24 well	1.9	0.4–1.6	500 µl	0.6–2.5	360 µl	200–800 ng	40 µl	2.0–3.2	360 µl	400 µl
12 well	3.8	0.8–3.2	1.0 ml	0.6–2.5	450 µl	250–1,000 ng	50 µl	2.5–4.0	450 µl	500 µl
6 well/35 mm	**9.2**	**2–8**	**2.0 ml**	**0.6–2.5**	**900 µl**	**0.5–2 µg**	**100 µl**	**5.0–8.0**	**900 µl**	**1 ml**
60 mm	21	4–16	4.0 ml	0.6–2.5	1.8 ml	1.0–4.0 µg	200 µl	10–16	1.8 ml	2 ml
100 mm	60	12–48	12 ml	0.6–2.5	6.3 ml	3.5–14 µg	700 µl	35–56	6.3 ml	7 ml

Table 3 provides recommendations for transfection of adherent and suspension cells grown in a number of standard tissue culture formats. The quantities given for the cell inoculum, as well as the amount of Cytofectene, plasmid, and media required for any given tissue culture plate may be substituted for those given for 6 well and 35 mm plates in the protocols in Section 2.3 (shown in bold in Table 3). The first line in Table 3 indicates the steps in the protocols where these substitutions should be made.

The volumes listed in this table are for transfection of each well in the format listed. Therefore, transfection of cells in all 48 wells of a 48 well plate would require between 48 and 77 µl of Cytofectene in total (48 x (1.0 to 1.6 µl)) and 4.8 to 19.2 µg of plasmid in total (48 x (100 to 400 ng). The total volume of the diluted DNA/Cytofectene solution required for transfection of all 48 wells is 9600 µl (48 x 200 µl).

The number of cells inoculated per dish has been adjusted in proportion to the surface area of the different-sized tissue culture dishes. The concentrations of the 10x Cytofectene/DNA solutions are the same in all formats (Sections 2.3 and 2.4, Steps 2 and 3). It is the volumes of these solutions that are adjusted in proportion to the surface area of the different-sized tissue culture dishes (Section 2.3, Step 4 and Section 2.4, Step 1). Therefore, the final concentrations of Cytofectene and plasmid are the same on all plates.

Section 4
References

1. Ausubel, F. M., *et al.*, eds, *Current Protocols in Molecular Biology,* Greene Publishing Associates and Wiley-Interscience, New York (1990).
2. Felgner, J. H., *et al.* Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations, *J. Biol. Chem,* **269**, 2550–2561 (1994).