



SureFECTOR™

Unit Size: 1 ml, 1mg/ml
Cat#: EM-101-001
Trial size set: 0.2 ml of each
UniFECTOR & SureFECTOR
Cat#: EM-101-003S

PRODUCT CHARACTERISTICS

SureFECTOR is a liposomal formulation of a polycationic lipid and a neutral, non-transfecting lipid compound. SureFECTOR has been used successfully to transfect DNA into HepG2, COS, PC12, NIH/3T3, Jurkat, primary rat and hamster hepatocytes, primary rat cardiomyocytes, primary male rat germ cells, primary chick retinal neurons, primary rat fibroblasts, primary quail myoblasts, primary human keratinocytes, and tracheobronchial cells. Peak activities were achieved using 1-4 μg SureFECTOR per μg DNA on the tested cell lines grown in 35 mm dishes. Therefore, 1 milliliter of SureFECTOR is sufficient for 250-1000 transfections. No significant toxicity was observed at the concentrations used for optimal activity on the cell lines tested. SureFECTOR is provided as a sterile suspension in water at a concentration of 1 mg/ml. Store at 4°C or above, DO NOT FREEZE.

OPTIMIZATION OF PARAMETERS

The conditions for optimal transfection efficiency vary between different cell types. To achieve the highest possible transfection efficiencies, several parameters need to be optimized. Once these parameters have been established for a particular cell line, reasonable reproducibility can be obtained from experiment to experiment. The following are the most important parameters:

Cell Confluence:

Cell confluence should be between 50-70%, and kept as constant as possible from experiment to experiment. In general, it is easier to control this parameter in medium to large wells (6 well plates, 35-60 mm culture dishes) than in smaller wells.

DNA/Liposome Complex:

The optimal ratio of DNA:liposome and the total amount of DNA/liposome complex should be determined using a constant number of cells to obtain the highest transfection efficiency. This is easily achieved starting with a constant amount of DNA (for example, 1 μg per 35 mm dish) and varying the amount of SureFECTOR (1-6 μg per dish in 0.5 or 1.0 μg increments). Then, keeping constant the DNA:liposome ratio that gave the highest efficiency in the previous experiment, vary the total amount of DNA/liposome complex and determine the new peak of activity. For example, if the DNA:liposome ratio in the first experiment was 1 $\mu\text{g}/1 \mu\text{g}$, then one could use 0.5 $\mu\text{g}/0.5 \mu\text{g}$, 1.0 $\mu\text{g}/1.0$



μg , 1.5 μg /1.5 μg and 2.0 μg /2.0 μg total amounts of DNA/liposome complex in the second experiment.

Transfection Time:

Transfection efficiency is related to the exposure time of the cells to the DNA/liposome complex. In general, the longer the exposure time the higher the efficiency. However, since transfection is carried out in serum-free or reduced serum medium, excessively long exposure times could lead to cell detachment or death. The recommended starting transfection time is 6-8 hour, however longer exposure times could prove optimal.

DNA TRANSFECTION OF ADHERENT CELLS (general protocol)

Cell plating:

The following protocol is for 35mm dishes. For other sizes, adjust the volumes proportionally. The cells are plated in six 35mm dishes at a concentration such that their confluency will be 50-70% when transfected (usually 24-48 hours after plating).

DNA/SureFECTOR complex:

NOTE: The DNA/liposome complex should be prepared at room temperature.

- Preparation of sterile DNA for transfections: Plasmid DNA (cesium chloride or similar purity grade) is ethanol precipitated in a sterile microfuge tube, washed with 70% ethanol, and dissolved in sterile deionized water to give a final concentration of 1 $\mu\text{g}/\mu\text{l}$. Store frozen at -20°C .
- Plasmid DNA (1-2 $\mu\text{g}/\text{dish}$) is diluted in sterile water or *serum and antibiotic-free* MEM to give a 1 $\mu\text{g}/100 \mu\text{l}$ final concentration
- SureFECTOR aliquots of 0, 1, 2, 4, or 6 μl are diluted with *serum and antibiotic-free* MEM to a final volume of 100 μl in siliconized microfuge tubes (see Note 1).
- The DNA is added directly to the diluted liposome solution with a pipettor and mixed by finger tapping the tubes or gently pipetting the liquid up and down.
- The DNA/liposome complex should form within seconds, however some investigators prefer to let the mixture sit for 15-45 minutes at room temperature (see Note 2).

Transfection:

- Wash the cells with *serum and antibiotic-free* MEM (2 x 1ml). Washing with PBS is not recommended since the residual phosphate from the PBS will compete with the DNA for the liposomes.
- Add 0.8 ml of *serum and antibiotic-free* MEM to each well containing the cells (see Note 3).
- Add the DNA/liposome complex solutions (200 μl) to the corresponding 35 mm wells in a dropwise fashion using a pipettor, trying to cover all areas of the well. Mix by gently rocking the plate(s).



- Incubate the cells for 5-18 hours under standard conditions (e.g., 37°C, 5% CO₂). 5-7 hours is a good starting range, however longer times may be used depending upon the cell type.
- Add 1 ml of MEM containing *twice as much FBS and antibiotics* as normally used to grow the cells. This attenuates the transfection and restores the serum and antibiotic concentrations to normal levels (see Note 4).
- Incubate the cells for an additional 18-24 hours under standard conditions.
- Replace the medium with complete medium.
- Assay the cells at the appropriate time (24- 72 hours) and determine the concentration range for peak expression of the reporter gene product. Narrow the concentration range for optimal activity as needed.

Note 1: Unsiliconized tubes can be used, but siliconized tubes appear to give better results.

Note 2: The DNA/liposome complex MUST be made in serum-free medium, otherwise negatively charged macromolecules in the serum will compete with the DNA for the liposome.

Note 3: If the cells require serum at all times for survival, 0.8 ml of MEM with reduced (20-50% of normal) serum, *but without antibiotics*, can be used instead. Antibiotics present during transfection can kill the cells and can decrease the transfection efficiency.

Note 4: For serum-free human keratinocytes, no FBS addition is needed. However, rinsing with medium containing BSA (0.5%) can be done to remove excess DNA/liposome complex. Then, serum-free medium is added to restore the normal culture conditions.

Transfections in the presence of serum (COS-1 cells in this example, but other cell lines also work with serum)

1. Make the DNA/liposome complex in *serum-free* MEM as above in 1 ml total volume and incubate at room temperature for 15 min.
2. Add 1 ml of MEM containing **20% FBS** to the above complex (final FBS concentration is 10%).
3. Add 2 ml/ea of the solution of step 2 to freshly aspirated 35 mm dishes containing COS-1 cells at 80% confluence.
4. Incubate cells overnight.
5. Aspirate the medium and replace it with 2ml/dish medium containing 10% FBS. Incubate for another 24h.
6. Rinse, fix and develop as normal.

Optimal conditions:

For a 35mm dish, the optimal DNA:SureFECTOR ratio is 4µg:20µg. For a serum-free experiment (control), the optimal ratio is 2µg:10µg.

DNA TRANSFECTION OF SUSPENSION CELLS

The following protocol is for 35 mm culture wells or dishes and has been shown to work well with Jurkat cells.



Preparation of cells:

- Transfer a stock suspension cell culture to a 50 ml conical tube and centrifuge at 400 x g for 10 min.
- Wash cells twice by aspirating the supernatant, gently resuspending the cell pellet in 10-20 ml of serum-free medium and centrifuging again at 400 x g for 10 min.
- Resuspend the pellet in serum-free medium to give a final concentration of 6×10^6 cells/ml.
- Plate six 35 mm culture wells or dishes with 0.8 ml of the cell suspension.

DNA/SureFECTOR complex:

- Prepare the DNA/liposome complex using the protocol described for adherent cells.

Transfection:

- Transfer the DNA/liposome complex to the cultures by randomly dropping the solution over the medium.
- Rock the wells gently to mix and incubate at 37°C for 5-8 hours or longer (see discussion of exposure time above).
- After 5-8 hours of incubation, add 4 ml growth medium containing 12.5% FBS to the wells and continue the incubation for an additional 72 hours.
- The cells can then be transferred to 10 ml Falcon tubes, the wells rinsed with 5 ml sterile PBS and added to the tubes, then pelleted at 400 x g for 10 min.
- The cell pellet is then washed twice with 5 ml sterile PBS and the final pellet resuspended in lysis buffer for assay of the gene product.