



siFECTOR™

Unit Size: 1 ml, 1mg/ml
Cat#: EM-101-005
Trial size: 0.2ml, 1mg/ml
Cat#: EM-101-004

PRODUCT CHARACTERISTICS

siFECTOR is a liposomal formulation of a polycationic lipid and a neutral, non-transfecting lipid compound. siFECTOR has been used successfully to transfect **siRNA** into CHO-K1, COS, NIH3T3, LNCaP, T-24, 293, C2C12, Schneider (Drosophila), SF-9, human bone marrow endothelial cells (HBMEC), primary rat aortic smooth muscle, primary rabbit myoblasts, and primary human keratinocytes. Peak activities were achieved using 2-6 μg siFECTOR per μg siRNA on the tested cell lines grown in 35 mm dishes. Therefore, 1 mL of siFECTOR is sufficient for 160-500 transfections. No significant toxicity was observed at the concentrations used for optimal activity on the cell lines tested. siFECTOR 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.

siRNA/Liposome Complex:

The optimal ratio of siRNA/liposome and the total amount of siRNA/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 siRNA (for example, 1 μg per 35 mm dish) and varying the amount of siFECTOR (1-6 μg per dish in 0.5 or 1.0 μg increments). Then, keeping constant the siRNA/liposome ratio that gave the highest efficiency in the previous experiment, vary the total amount of siRNA/liposome complex and determine the new peak of activity. For example, if the siRNA/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



$\mu\text{g}/1.0 \mu\text{g}$, $1.5 \mu\text{g}/1.5 \mu\text{g}$ and $2.0 \mu\text{g}/2.0 \mu\text{g}$ total amounts of siRNA/liposome complex in the second experiment.

Transfection Time:

Transfection efficiency is related to the exposure time of the cells to the siRNA/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.

siRNA 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).

siRNA/siFECTOR complex:

The siRNA/liposome complex should be prepared at room temperature.

Preparation of siRNA for transfections:

siRNA is preferably chemically synthesized using appropriately protected ribonucleoside phosphoramidites. Dharmacon Inc. provides the siRNA as a purified duplex with a purity >97%. Redissolve the shipped RNA duplex pellet in water and continue directly with transfection (see below). In our experience, Dharmacon provides the highest quality siRNA on the market. siFECTOR 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 siRNA solution is added directly to the diluted liposome solutions with a pipettor and mixed by finger tapping the tubes or gently pipetting the liquid up and down. The siRNA/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 siRNA for the liposomes.
- Add 0.8 ml of *serum and antibiotic-free* MEM to each well containing the cells (see Note 3).
- Add the siRNA/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 1ml 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 siRNA/liposome complex MUST be made in serum-free medium, otherwise negatively charged macromolecules in the serum will compete with the siRNA 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 siRNA/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 siRNA/liposome complex in *serum-free* MEM as above in 1 ml total volume 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 siRNA:siFECTOR ratio is 4µg:20µg. For a serum-free experiment (control), the optimal ratio is 2µg:10µg.