

Product Information

ESCORT™III Transfection Reagent

Product No. **L3037**

Product Description

ESCORT™ III is a liposome suspension composed of a polycationic lipid and a neutral, non-transfecting lipid compound. ESCORT III has been used successfully to transfect DNA into a variety of cell types (see Table below). Peak activities were achieved using 1-4 µg ESCORT III per µg DNA on the tested cell lines grown in 35 mm dishes. One ml ESCORT III is sufficient for 250-1000 transfections. No significant toxicity was observed at the concentrations used for optimal activity on the cell lines tested. ESCORT III is provided as a sterile suspension in water at a concentration of 1 mg/ml.

Transfected Cell Types	
HepG2 COS PC12	NIH3T3 Jurkat
Primary cell types: rat and hamster hepatocytes rat cardiomyocytes male rat germ cells chick retinal neurons rat fibroblasts quail myoblasts human keratinocytes human tracheobronchial cells	

Preliminary Considerations

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**
Cells should be between 50-70% confluent 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 sizes.
- DNA/liposome ratio**
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 (e.g. 1 µg per 35 mm dish) and varying the amount of ESCORT III (1-6 µg per dish in 0.5 or 1.0 µg increments). In the next experiment use the optimal DNA/liposome ratio, varying 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 is 1 µg/1 µg, then one could use 0.5 µg/0.5 µg, 1.0 µg/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**
In general, the longer the transfection time, the higher the efficiency. For transfections carried out in serum-free or reduced-serum medium, excessively long transfection times could lead to cell detachment or death. The recommended starting transfection time is 6-8 hours; however, longer exposure times could prove optimal.

Precautions and Disclaimer

Sigma's ESCORT III transfection reagent is for laboratory use only. Not for drug, household, or other uses.

Storage

Store at 2-8°C. DO NOT FREEZE.

Procedures

A. Procedure for DNA Transfection of Adherent Cells

The following protocol is for 35 mm dishes, for other sizes, adjust the volumes proportionally.

Preparation of cells:

1. Culture cells so that confluency will be 50-70% in 24-48 hours.

Preparation of ESCORT III/DNA complexes:

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

2. Ethanol precipitate the plasmid DNA (cesium chloride or similar purity grade), wash once with 70% ethanol, and dissolve in sterile deionized water to give a final concentration of 1 µg/µl. Store at -20°C.
3. Dilute the plasmid DNA (1-2 µg/dish) in sterile water or serum-free and antibiotic-free MEM to give 1 µg/100 µl final concentration.
4. Dilute 1, 2, 4, and 6 µl aliquots of Escort III (plus a negative control) to a final volume of 100 µl with serum-free and antibiotic-free MEM in siliconized microcentrifuge tubes. Unsiliconized tubes can be used, but siliconized tubes appear to give better results.
5. Add the plasmid DNA solution (100 µl) directly to the diluted liposome solutions (100 µl) and gently mix by finger tapping the tubes or pipetting the liquid up and down.
6. Allow the DNA/liposome complexes to form. Complexes should form within seconds, however, 15-45 minutes at room temperature is recommended. Please note that 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 liposomes.

Transfection:

7. Wash the cells twice with 1 ml of serum-free and antibiotic-free MEM. Caution: Washing with PBS is **not** recommended, as the residual phosphate from the PBS will compete with the DNA for the liposomes.
8. Add 0.8 ml of serum-free and antibiotic-free MEM to each well containing the cells. 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 of serum-free and antibiotic-free MEM. Antibiotics present during transfection can kill the cells and can decrease the transfection efficiency.
9. Add the DNA/liposome complex solution (200 µl) to the corresponding 35 mm wells in a dropwise fashion, using a pipet in order to cover all areas of the well. Mix by gently swirling the plates.
10. Incubate the cells for 5-18 hours under standard growing conditions. Five to seven hours is a good starting range, however longer times may be required for optimal transfection.
11. Add 1 ml of complete MEM containing twice as much serum and antibiotics as normally used to grow the cells. This attenuates the transfection and restores the serum and antibiotic concentrations to normal levels. For serum-free human keratinocytes, no serum addition is needed. Excess lipid/DNA complexes can be removed by rinsing with medium containing BSA (0.5%). If BSA is added to the medium used for rinsing, serum-free medium should then be added to restore the normal culture conditions.
12. Incubate the cells for an additional 18-24 hours under standard conditions.
13. Replace the MEM medium with complete medium.

Detection:

14. Assay the cells at 24 to 72 hours post-transfection. Determine the concentration range for peak expression of the reporter gene product. Narrow the concentration range to determine the optimal activity.

B. 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:

1. Transfer a suspension cell culture to a 50 ml conical tube and centrifuge at 400 X g for 10 minutes.
2. Aspirate the supernatant and gently suspend the cell pellet in 10-20 ml of serum-free medium. Centrifuge at 400 X g for 10 minutes. Repeat this step once.
3. Suspend the pellet in serum-free growth medium to give a final concentration of 6×10^6 cells /ml.
4. Divide cells among six 35 mm culture wells or dishes with 0.8 ml of the cell suspension per well/dish.

Preparation of ESCORT III/DNA complexes:

5. Prepare the DNA/liposome complexes using Steps 2-6 in the protocol described for adherent cells.

Transfection:

6. Transfer the DNA/liposome complex to the cell cultures dropwise using a pipetter.
7. Swirl the wells gently to mix and incubate at 37°C for 5-8 hours or longer.
8. After a 5-8 hour incubation, add 4 ml growth medium containing 12.5 % serum to the wells and continue the incubation for an additional 72 hours.
9. Transfer the cells to a 10 ml centrifuge tube. Add 5 ml sterile PBS to rinse the wells and transfer the wash to the 10 ml centrifuge tube.
10. Centrifuge at 400 X g for 10 minutes.
11. Wash the cell pellet twice as in steps 9 and 10 with 5 ml sterile PBS.

Detection:

12. Assay the cells at 24 to 72 hours post-transfection. Determine the concentration range for peak expression of the reporter gene product. Narrow the concentration range to determine the optimal activity.

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