Product Information

ESCORT™ IV Transfection Reagent

L3287

Product Description

ESCORT™ IV is composed of a polycationic lipid and a neutral, non-transfecting lipid, and has been used successfully to transfect DNA into a variety of cell types (see Table below). This reagent has also been successfully tested for RNA transfections. Peak activities were achieved using 2-6 µL ESCORT™ IV per µg DNA on the tested cell lines grown in 35 mm dishes. One mL ESCORT™ IV is sufficient for 160-500 transfections. This reagent also works in medium containing 10% fetal bovine serum.
No significant toxicity was observed at the concentrations used for optimal expression on the cell lines tested. ESCORT™ IV is provided as a sterile suspension in water at a concentration of 1 mg/mL.

Transfected Cell Types

CHO-K1	C2C12	Human Bone Marrow Endothelial Cells (HBMEC)
COS1	Schneider (Drosophila)	Primary Rat Aortic Smooth Muscle
COS7	SF-9	Primary Rabbit Myoblasts
NIH3T3	LNCaP	Primary Human Keratinocytes
HEK293	HeLa	T24

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, good reproducibility can be obtained from experiment to experiment. The following are the most important parameters.

Cell Confluence

Cells should be between 50-70% confluent at the time of transfection. This parameter should be kept as consistent as possible from experiment to experiment. In general, this is easier to control 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 consistent cell density 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 ESCORT™ IV (1-6 µg per dish in 0.5 or 1.0 µg increments). In the next experiment use the optimal DNA/liposome ratio and 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 1.0 µg/1.0 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 exposure time the higher the efficiency. For transfections 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 hours, however longer exposure times could prove optimal.

Precautions and Disclaimer

Our ESCORT™ IV transfection reagent is for laboratory use only. Not for drug, household, or other uses.

Storage

Store at 2-8 °C. DO NOT FREEZE.

Procedure

DNA Transfection of Cells in Serum-Free Medium

Preparation of cells

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

1. Culture cells to achieve 50-70% confluency at the time of transfection (in 24-48 hours). Preparation of the DNA/ESCORT™ IV complex: 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 DNA samples frozen at minus 20 °C.).
3. Dilute the plasmid DNA (1-2 µg/dish) in serum-free and antibiotics-free MEM to give 1 µg/100 µL final concentration.
4. Dilute 1, 2, 4, or 6 µL aliquots of ESCORT™ IV to a final volume of 100 µL with serum-free and antibiotics-free MEM. Include a negative control without ESCORT™ IV.

   Note: Unsiliconized tubes can be used, but siliconized tubes appear to give better results.
5. Add the plasmid DNA solution directly to the diluted liposome solutions 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.

   Note: 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.

Transfection

7. Wash the cells twice with 1 mL volumes of serum 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- and antibiotics-free MEM to each well containing the cells.

   Note: 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.
9. Add the DNA/liposome complex solutions (200 µL) to the corresponding 35 mm wells in a dropwise manner, trying to cover all areas of the well. Mix by gently swirling the plates.
10. Incubate the cells for 5-18 hours under standard culture conditions. Five to seven hours is a good starting range, however longer times may be required for optimal transfection efficiency.
11. Add 1 mL of complete MEM (with serum and antibiotics) 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.

    Note: For serum-free human keratinocytes, no serum addition is needed. However, excess DNA/liposome complexes can be removed by rinsing with medium containing BSA (0.5%) if necessary, followed by the addition of serum free medium to restore the normal culture conditions.
12. Incubate the cells for additional 18-24 hours under standard growth conditions.
13. Aspirate the medium and replace it with complete medium.
14. Assay the cells at the appropriate time (24 to 72 hours post-transfection) and determine the concentration range for peak expression of the reporter gene product. Narrow the concentration range for optimal activity as needed.

DNA Transfection of Adherent Cells in the Presence of Serum

1. Prepare the DNA/liposome complex in serum-free MEM in 1 mL total volume at room temperature for 15 minutes (Follow Steps 2-6 above for suspension cells).
2. Add 1 mL of MEM containing 20% fetal bovine serum to the above complex (final FBS concentration is 10%).
3. Remove the culture medium from the growing cells. Immediately add 2 mL of the DNA/liposome/MEM solution (step 2) to the freshly aspirated 35 mm dishes at 80% confluence.
4. Incubate cells overnight.
5. Aspirate the medium and replace it with 2 mL medium containing 10% serum. Incubate for another 24 hours.

6. Assay the cells for expression at the appropriate time (24 to 72 hours post-transfection) and determine the concentration range for peak expression of the reporter gene product. Narrow the concentration range for optimal activity as needed. Optimal conditions: For a 35 mm dish, the optimal DNA/ESCORT™ IV amounts are 4 µg/20 µg. Under serum-ree conditions (control), the optimal values are 2 µg/10 µg.

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