

Product Information

ESCORT[®] Transfection Reagent

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Product Description

ESCORT[™] is a very efficient transfection reagent, suitable for use in transfecting nucleic acids into eukaryotic cells grown in culture. ESCORT is a liposome formulation of the cationic lipid N-[1(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTAP) and dioleoyl phosphatidylethanolamine (DOPE)¹ present at a ratio of 1:1 (w/w). ESCORT is hydrated in 0.2 µm filtered 20 mM MES, 150 mM NaCl, pH 6.2, and is aseptically filled. The concentration of total lipid in ESCORT is 2 mg/ml.

Reagents and Equipment Required but Not Provided

- DME medium (DMEM) without supplements, Product No. D5671
- DME medium supplemented with 10% bovine calf serum, 4 mM glutamine, penicillin G/streptomycin (10,000 units Penicillin G and 10 mg streptomycin/ml, Product No. P0781) diluted 1:100 in DMEM (complete medium)
- Glass tubes 12X75mm or 13X75mm, sterile

Precautions and Disclaimer

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

Storage

Store at 2-8 °C

Procedure

Transfection Efficiency

Using HeLa cells and pSV40-CAT reporter gene, 5 µl of ESCORT is sufficient for transfection of cells on 35 mm culture dishes and 15 µl is sufficient for 60 mm culture dishes. The volume required for transfection may vary from cell line to cell line. The procedure stated below is designed for HeLa cells using the pSV40-CAT plasmid at 1 µg/µl (diluted in sterile deionized water). The absolute efficiency of ESCORT is very dependent on the condition of the HeLa cells, the duration of treatment with the ESCORT-DNA complex, and other factors.

Note: Culture HeLa cells in DMEM supplemented with 10% bovine calf serum, 4 mM glutamine, 1:100 penicillin/streptomycin. Perform the following using aseptic techniques:

A. Plate the cells for transfection experiment:

1. Two days before the experiment split cells 1:1.
2. One day (20-24 hr.) before the experiment seed cells at a concentration of 2×10^5 per 35 mm dish (use 6 well plate), or 6×10^5 per 60 mm dish.

B. HeLa cells transfection

Note: Gently mix ESCORT just prior to use.

1. Prepare 250 µl of ESCORT-DNA mixture as follows:

15 µl of ESCORT
230 µl DMEM (without supplements)
5 µl of 1 µg/µl pSV40-CAT DNA (5 µg total)

Mix by pipetting.

2. Incubate for 15 minutes at room temperature.
3. Near the end of the DNA/ESCORT incubation, prepare the cells for transfection by removing the medium from the cell culture dishes.
4. Add 2 ml of complete medium to the ESCORT-DNA mixture.
5. Mix by pipetting.
6. Add the ESCORT-DNA medium mixture to the cells. For 35 mm dishes, add 0.7 ml/dish in duplicate or triplicate. For 60 mm dishes, add the entire mixture (2.25 ml) to one dish.

7. Incubate the cells for 5-6 hours in a cell culture incubator (37 °C).
8. Following the incubation, aspirate the medium containing ESCORT-DNA and add 2 ml of complete medium to the 35 mm dishes or 5 ml to the 60 mm dishes. If working with 60 mm dishes, it is preferable to dilute the ESCORT-DNA in the culture dish four-fold with complete medium, without aspiration.
9. Incubate the cells for another 40 hours in a cell culture incubator (37 °C).
10. Lyse the cells and assay for CAT protein.

The yield of CAT protein is approximately 0.6 ng/35 mm dish and 2 ng/60 mm dish.

Reference

Leventis, R. And Silviu, J.R., *Biochim. Biophys. Acta*, **1023**, 124, (1990)

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