3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

ESCORT™ II Transfection Reagent
Product No. **L 6037**

Product Description

ESCORT II ™ transfection reagent is a unique formulation of the neutral lipid dioleoyl phosphatidyl-ethanolamine (DOPE) and a proprietary cationic lipid. Each ESCORT II reagent set includes DNA diluent buffers that increase transfection efficiency in the presence of serum.

Reagents Provided

0.75 ml ESCORT II

- ESCORT II, Product No. L 6037 0.75 ml
- Hydration Buffer, Product No. B 1180 0.8 ml
- DNA Diluent, Product No. D 6813 4 ml

Sufficient for 75 transfections using 2 µg of DNA per well

1.5 ml ESCORT II

- ESCORT II, Product No. L 6037 1.5 ml
- Hydration Buffer, Product No. B 1180 1.6 ml
- DNA Diluent, Product No. D 6813 8 ml

Sufficient for 150 transfections using 2 µg of DNA per well

Transfected Cell Types

ESCORT II reagent has been successfully used to transfect β-galactosidase reporter gene into the cell lines listed below.

Transfected Cell Types	
HeLa S3	**BHK-21**
HEK 293	**CHO-K1**
MDCK	**CV1**
NIH 3T3	**COS-1**
B16-F0	**COS-7**
PC-12	**HepG2**
K562	**P19**
HeLa	**HUVEC-C**

Preliminary Considerations

The conditions for optimal transfection efficiency vary between different cell types. To achieve the highest possible transfection efficiencies, several parameters must 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 quantity and DNA/Liposome ratio

Using the DNA quantities and DNA/liposome ratios recommended in Tables 1, 2, and 3 will work with a majority of cell types. However, to obtain the maximum transfection efficiency for a particular cell type, the optimal ratio of DNA/liposome and the total amount of DNA/liposome complex should be determined using a consistent cell density. To determine the optimal ratio and the total amount of DNA, perform a trial transfection while maintaining the suggested fixed ratio of ESCORT II reagent to DNA and varying the DNA quantity over the suggested range (Table 2). If necessary, optimize the ratio of ESCORT II reagent to DNA by using 3 to 6 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized.

Precautions and Disclaimer

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

Storage and Stability
Store at 2-8°C. **DO NOT FREEZE.**

Dried ESCORT II reagent is stable for at least 1 year at 2-8°C. Hydrated ESCORT II reagent is stable for at least 6 months at 2-8°C. DNA diluent is stable for at least 6 months at 2-8°C.

Preparation Instructions
1. Hydrate the ESCORT II lipid film at room temperature with 0.75 ml or 1.5 ml of Hydration Buffer respectively for the 0.75 or 1.5 ml package of ESCORT II. Vortex for 10 seconds at top speed before use. Store the hydrated ESCORT II reagent at 2-8 °C and vortex briefly before each use**.**
2. Use the DNA diluent provided to prepare the DNA solution. Use 25 µl of diluent for 1 µg DNA. Avoid vortexing the DNA diluent solution.
3. For most cell types, use 5 µl of ESCORT II reagent with 1 µg of DNA.

Procedure
A. DNA Transfection of Adherent Cells
1. Dilute the hydrated ESCORT II reagent with serum-free medium with the volume indicated in Table 1.

Table 1. Dilution Table for ESCORT II

Serum Free Medium (µl)	ESCORT II (µl)
10	2.5
20	5
40	10
80	20
160	40

2. Dilute the DNA with the DNA diluent with the volume indicated in Table 2, and incubate 1 to 5 minutes at room temperature. **Do not incubate longer than 5 minutes.** Avoid vortexing the DNA diluent.

Table 2. Dilution Table for DNA

DNA (µg)	DNA Diluent (µl)
0.5	12.5
1	25
2	50
4	100
8	200

3. Add the DNA solution to the diluted ESCORT II reagent. Incubate at room temperature for 5 to 10 minutes to form ESCORT II/DNA complexes. **Do not incubate longer than 30 minutes.**

4. Add the mixture of ESCORT II/DNA complexes directly to the cells growing in serum-containing culture medium. Refer to Table 3 for appropriate transfection volumes. Incubate at 37 °C.

Table 3. Usage of DNA/ESCORT II Complexes

Tissue culture dish/well	DNA (µg)	ESCORT II/ DNA complex (µl)	Medium in wells (µl)
96-well	0.1-0.5	5-25	95-75
24-well	0.5-2	25-100	225-150
6-well	2-6	100-300	900-700
60 mm	6-8	300-400	2200-2100
100 mm	8-12	400-600	4600-4400

Note: For some cells such as HeLa-S3, MDCK or CHO-K1, higher transfection efficiencies can be achieved when an initial 4-hour incubation is done in serum-free media. After this step, add one volume of medium containing 20% serum, and then proceed as in Step 5.

5. After 24 hours post transfection, add fresh growth media as needed. For some cell types, the old media can be removed and replaced with fresh media at this step.

6. Assay for the reporter gene can be performed 24 to 72 hours following transfection as appropriate.

Note: The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

B. DNA Transfection of Suspension Cells

For suspension cells, the basic protocol and optimization protocol is the same as described for adherent cells, with the following exceptions:

1. The day before transfection, split the cells so they are in good condition on the day of transfection.

2. While the ESCORT II/DNA complexes are incubating, spin down the cells, suspend them at 1 x 10^6 or 2 x 10^6 cells/ml in medium either with or without serum, and transfer the appropriate volume indicated in Table 4 to the dish.

Table 4. Usage of DNA/ESCORT II Complexes for Suspension Cells

Tissue culture dish/well	Cell count (x 10^6)	ESCORT II/DNA complex (µl)	Medium in wells (µl)
96-well	0.1	5-25	95-75
24-well	0.5	25-100	225-150
6-well	2	100-300	900-700
60 mm	5	300-400	2200-2100
100 mm	10	400-600	4600-4400

3. Prepare the mixture of ESCORT II/DNA complexes as in protocol I, add it directly to the cells, and mix well by gently pipetting 2 to 3 times. Incubate at 37 °C and proceed as described for adherent cells.

Notes:

- This step is important because some suspension cells have a tendency to clump, and the reagent does not easily access cells in the center of clumps. Gentle pipetting of cells disrupts these clumps and produces a true single cell suspension, which will increase transfection efficiency.

- For some hematopoietic cell lines, mitogenic agents like PHA or PMA may be added to the cells 4 hours after transfection to a final concentration of 1 mg/ml or 50 ng/ml, respectively, to enhance the levels of gene expression.

- Although ESCORT II reagent works well for cells such as K562 and PC-12, which can grow in suspension, it does not work well for Jurkat cells. For transfection of Jurkat cells, we recommend using the ESCORT III (Product No. L 3037) reagent.

C. Low Quantity DNA Transfection

The following revised[1,2] protocol can be used to facilitate pipetting and transfer of DNA/lipids complexes to the cells when a low quantity of DNA (< 1 µg) is used for the transfection.

1. Dilute hydrated ESCORT II reagent with serum-free medium as indicated in Table 5.

Table 5. Recommended Amounts of Reagents for Low Quantity DNA Transfection

DNA (µg)	Serum-free medium (µl)	ESCORTII/DNA complex (µl)
0.125	49.37	0.63
0.25	48.75	1.25
0.5	47.5	2.5
1	45	5

2. First dilute the DNA diluent in serum-free medium and then add the DNA. See Table 6 for volumes of serum-free medium, DNA diluent, and DNA amount. Incubate 1 to 5 minutes at room temperature.

Table 6. DNA Dilution for Low Quantity DNA Transfection

Serum-free medium (µl)	DNA Diluent (µg)	DNA (µg)
46.8	3.12	0.125
43.75	6.25	0.25
37.5	12.5	0.5
25	25	1

3. Proceed as in Steps 3 though 5 as described for adherent cells. See Table 7 for appropriate transfection volumes.

Table 7. Usage of DNA/ESCORT II Complexes in Low Quantity DNA Transfection

Tissue culture dish/well	DNA diluent (µg)	ESCORT II/DNA complex (µl)	Medium in wells (µl)
96-well	0.1-0.25	50	Aspirate media before adding complex
24-well	0.5-2	50-100	200-150

References

1. Felgner, J. H., et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J. Biol. Chem., **269**, 2550-2561 (1994).

2. Felgner, P. L., et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. Proc. Natl. Acad. Sci. USA, **84,** 7413-7417 (1987).

SU 09/03