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ProductInformation

ESCORT™ V Transfection Reagent

Catalog Number **E9778**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description

ESCORT V Transfection Reagent was developed for highly efficient stable and transient transfections of eukaryotic cells. It is a specially processed polyethyleneimine (PEI) optimized for use in serum-containing media. Transfection in serum-free media has been demonstrated, and Escort V can also be used for transfection experiments with siRNA.

The reagent has been extensively tested and found to be highly efficient in many cell types including CHO, BHK, HeLa, HEK293, HEK-293T, Vero, F9, PC12, COS-1, COS-7, NIH 3T3, L929, Human Foreskin Fibroblasts (HFF) primary cells, and Bovine Aorta Endothelial cells (BAEC).

Reagents

ESCORT V Transfection Reagent, Catalog Number E0654	1.5 ml
ESCORT V Transfection Buffer, Catalog Number E0529	100 ml

ESCORT V Transfection Reagent is provided at a concentration of 1.3 mg/mL. The reagent is sufficient for 700-1000 transfection experiments in 24-well plates.

Precautions and Disclaimer

These products are for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C.

PROCEDURE

This protocol is optimized for use in 24-well plates. All volumes given are for a single reaction (single well) on a 24-well plate. If another format is desired, scale up or down accordingly (Please see Table I).

Cell Preparation

Adherent cells

1. 18-24 hours before transfection, seed the plate with 5-7.5 x 10^4 cells/well in 0.5 ml of the appropriate medium.
2. 30 minutes to 2 hours before adding the transfection cocktail to the wells, replace the growth media in the wells with 0.5 ml fresh media containing serum (complete media).

Cells in suspension

1. Before transfection, pellet the cells and dilute to the appropriate concentration (0.5-1 x 10^6 cells/ml) in the standard growth medium for the cell line.
2. Add 0.5 ml of the cell suspension to each well.

Transfection with plasmid DNA in 24-well plate

For optimal transfection, the recommended ratio of µg DNA/µL ESCORT V Transfection Reagent is 1:3. In some cases, transfection efficiency can be increased by using a different ratio. When changing the amount of ESCORT V Transfection Reagent (step 2), keep the amount of Transfection Buffer constant at 60 µL.

1. In a sterile tube, combine 60 µl of ESCORT V Transfection Buffer and 0.7 µg of plasmid DNA. Mix the contents of the tube gently.
2. In a separate tube, combine 60 µl of ESCORT V Transfection Buffer and 2.1 µl of Escort V Transfection Reagent. Mix gently.
3. Combine the DNA/buffer solution, Step 1, with the ESCORT V reagent/buffer solution, Step 2, to make the transfection cocktail. Mix gently.
4. Incubate at room temperature for 15-20 minutes. The complex is stable for up to 2 hours at room temperature.
5. Add the total volume of transfection cocktail directly to the wells. Mix gently by rocking the plate.
6. Incubate the transfected cells under standard conditions for 24-72 hours.

Stable Transfection

1. Perform cell transfection using the appropriate vector. Follow the protocol above.
2. Two days after transfection, replace the media with fresh media containing the appropriate selection antibiotic.
3. Maintain the cells in selective media for 2-3 weeks until stably transfected cells are clearly identified.

Transfection with siRNA in a 6-well plate

This protocol is optimized for cells plated in a 6-well plate. Volumes indicated in the protocol are for one reaction (one well).

1. Prepare 10 nmol siRNA stock solution (dilute with RNAse-free water).
2. In a sterile tube, add 60 µL of ESCORT V Transfection Buffer and 9 µL of ESCORT V Transfection Reagent.
3. Mix the contents of the tube gently and incubate for 5 minutes at room temperature.
4. In a second sterile tube, add 60 µL of serum-free medium and 10 µL of 10 nmol siRNA solution.
5. Combine the contents of both tubes and gently mix.
6. Incubate for 15-20 minutes at room temperature to form the transfection complex.
7. During incubation, remove culture media from the wells and add 0.8 ml of complete culture media (media with serum) to each well.
8. Carefully add the transfection mix drop-wise to the cells in the well.
9. Swirl the plate gently to ensure uniform distribution of the transfection complex.
10. Incubate the cells at 37 °C in 5% CO_2 for 24-72 hours before analysis.
11. Change the media as required.

Transfection with siRNA in a 96-well plate

This protocol is optimized for a 96-well plate. Volumes indicated in the protocol are for one reaction (one well).

1. Prepare 100 pmol siRNA stock solution (dilute with RNAse-free water).
2. In a sterile tube, add 25 µL of ESCORT V Transfection Buffer and 0.6 µL of ESCORT V Transfection Reagent.
3. Mix the contents of the tube gently and incubate for 5 minutes at room temperature.
4. In a second sterile tube, add 25 µL of serum-free media and 10 µL of the 100 pmol siRNA solution.
5. Combine the contents of both tubes and mix carefully.
6. Incubate for 10-15 minutes at room temperature to form the transfection complex.
7. During incubation, remove the culture media from the wells and add 100 µL of complete culture media (media with serum) to each well.
8. Carefully add the transfection mix drop-wise onto the cells in the well.
9. Swirl the plate gently to ensure uniform distribution of the transfection complex.
10. Incubate the cells at 37 °C in 5% CO_2 for 24-72 hours before expression analysis.
11. Change the media as required.

OPTIMIZING TRANSFECTION

For some cell lines, ESCORT V Transfection Reagent may require optimization. When optimizing conditions, it is important to consider the following optimization factors:

Volume of Transfection Cocktail

In some cases, a different volume of the transfection mixture may increase transfection rates. For optimization, compare transfection performance when different volumes of transfection mixture are added to the wells (e.g., 75, 100, 120, and 150 µL/well).

The Ratio of DNA/ESCORT V

The recommended ratio of µg DNA/ µl ESCORT V Transfection Reagent is 1:3. This ratio produces good results for most cell lines. In some cases, transfection efficiency can be increased by changing the ratio (e.g., 1:2 or 1:4).

Media

It is recommended to change growth media up to 2 hours before transfection. Use complete media (media supplemented with serum). If desired, media containing the transfection mixture can be substituted 4-6 hours after the initial addition without loss of transfection activity.

Transfection without ESCORT V Transfection Buffer

If use of the ESCORT V Transfection Buffer is not desired, prepare the transfection mixture in any standard media without serum and antibiotics. Follow the same volumes as in transfection protocol.

Cell Density
The optimal number of cells to be plated depends on the specific cell line. A 40%-70% confluent cell layer at the time of transfection is suggested for best response. When required, cells can be transfected at very low cell densities such as 5-15% confluence.

Incubation Time Post-Transfection
Incubation time depends on the cell line, the protein being expressed, as well as the vector construct.

DNA Quality
DNA quality is a critical factor for successful transfection. $OD_{260/280}$ should be ~1.8 or greater (1.85 is recommended). DNA should be free of endotoxin and other contaminants. RNA contamination does not prevent transfection; however, RNA substitutes DNA in the complex and may lead to an incorrect DNA concentration estimation. Taken together, these factors may greatly reduce transfection efficiency.

DNA Vector
The expression of the transfected gene depends on the cell line, the promoter used, and the nature of the expressed protein.

Condition of the Cells
Cells should be healthy, free of contamination, proliferating well and plated at an appropriate density.

The Level of the Expressed Gene
High level of expression of some proteins can be cytotoxic.

Table I Scaling up/down

Transfection vessel	Well diameter (mm)	Growth area (cm^2)	Factor to 24-wells	Media volume (ml)	DNA/ESCORT V cocktail to add
100 mm	100.00	78.50	41.00	21.00	3 - 8 ml
60 mm	60.00	30.00	14.70	8.00	1 - 3 ml
35 mm	35.00	9.50	5.00	2.50	0.38 -1 ml
6-well	34.80	9.50	5.00	2.50	0.38 -1 ml
12-well	22.10	3.80	2.00	1.00	150 - 400 μL
24-well	15.60	1.90	1.00	0.50	70 – 200 μL
48-well	10.20	0.80	0.42	0.21	30 – 80 μL
96-well	6.40	0.32	0.17	0.08	13 - 34 μL

Troubleshooting Guide

Problem	Possible cause	Suggestions
Low transfection efficiency	Amount of DNA/ESCORT V cocktail	Optimize according to the comments in the "Optimizing Transfection" section.
	Contaminated DNA	Use a high-quality plasmid preparation method and see that the OD ratio 260/280 is 1.8 – 1.85.
		Use endotoxin free DNA (for endotoxin removal use Endotoxin Removal Solution, Catalog Number E4274.
	Suboptimal DNA/ESCORT V Transfection Reagent ratio	Optimize according to the comments in the "Optimizing Transfection" section.
	Vector used	In order to achieve an optimal expression rate of the transfected gene, the promoter should be compatible with the cell line.
		Low transfection efficiency results in low expression rates. On the other hand, very high exogenous protein expression levels may be cytotoxic
		Perform a control transfection
	Cell growth conditions	If cells have a high passage number, start a new culture from stocks of a lower passage number
		See that cells were not dramatically stressed during plating procedure or while incubated. See that the medium and serum used are optimal for cell growth.
		Check for the presence of mycoplasma in the cells
		Check that the cells are plated at the optimal density
	Assay	Use a positive control to ensure that the assay works properly.
Signs of cell cytotoxicity	Expressed protein is toxic to the cells at the current expression level	If the particular cell line is obligatory, try to express the gene under a different promoter.
	Amount of DNA/ESCORT V cocktail	Optimize according to the comments in the "Optimizing Transfection" section. Substitute the media containing the transfection cocktail with fresh media 6-24 hours post transfection.
	Contaminated DNA	Use a high-quality plasmid vector.
		Use endotoxin free DNA (for endotoxin removal use Endotoxin Removal Solution, Catalog Number E4274.
	Cells are stressed	See that cells were not dramatically stressed during plating procedure or while incubated.
	Mycoplasma contamination	Check for the presence of mycoplasma in the cells.
Transfection efficiency varies between repeats within the same experiment	Cells density and incubation conditions	The density of the cells in the different wells might vary due to clump formation or cells seeding without mixing. Avoid clump formation following trypsinization by repeated cells pipetting. See that the plate placed in the incubator is perfectly horizontal and not adjacent to the incubator well.
	Mycoplasma contamination	Prepare new cells.
	Cell passage number is too high	Prepare new cells.

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