LyoVec™

A lyophilized cationic lipid for transfection of mammalian cells

Catalog # lyec-12, lyec-22

For research use only

Version # 16B18-MM

PRODUCT INFORMATION

Contents:

LyoVec™ is supplied as a sterile lyophilized lipid formulation to be reconstituted with water. LyoVec™ is compatible with all cell culture media. After reconstitution, 50 µl of LyoVec™ is recommended per transfection of 1-4 x10^5 cells/well in a 12-well plate.

- lyec-12: 4 vials, sufficient to perform 160 reactions
- lyec-22: 9 vials, sufficient to perform 360 reactions

where one reaction is a transfection of 1-4 x 10^5 cells/well in a 12-well plate.

LyoVec™-GFP/SEAP is a ready-made lipid-DNA lyophilized complex provided as a transfection control. The complex combines LyoVec™ and a GFP/SEAP reporter plasmid to assess transfection efficiency by GFP fluorescence and/or secreted alkaline phosphatase (SEAP) staining. After reconstitution, 50 µl of LyoVec™-GFP/SEAP is recommended per transfection of 1-4 x10^5 cells/well in a 12-well plate.

- lyec-ls: 1 vial, sufficient to perform 20 reactions.

Storage and stability:

LyoVec™ and LyoVec™-GFP/SEAP are shipped at room temperature and should be stored at 4 °C. Lyophilized products are stable for 1 year when properly stored. After resuspension, store products at 4 °C. Resuspended LyoVec™ is stable for 6 months and resuspended LyoVec™-GFP/SEAP is stable 2 months.

Quality control:

- Transfection efficiency of LyoVec™ is evaluated with 293 cells. The cells are transfected with LyoVec™/DNA complexes prepared by mixing 1 µg of a GFP/SEAP plasmid with 100 µl of LyoVec™. The amount of SEAP is quantified 48 h after transfection using QUANTI-Blue™. The percentage of green cells 72 h after transfection is determined by FACS. Typically, >80% of cells are transfected.
- Cytotoxicity of LyoVec™: the survival rate of 293 cells exposed to LyoVec™ for 72 hours in medium containg 10% serum is >95%.

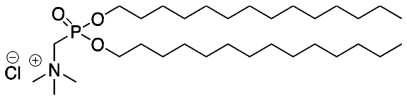
DESCRIPTION

LyoVec™ is a novel proprietary cationic lipid belonging to the new family of phosphonolipids originally described by Floch et *al.*, as efficient transfection reagents both *in vitro* and *in vivo*[1-2]. Their positive charge enables them to bind to DNA, and their phospholipid structure promotes fusion with cellular membranes for DNA delivery.

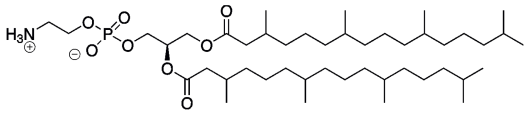
1. Floch *et al.* 1997. Cationic phosphonolipids as non viral vectors for DNA transfection in hematopoietic cell lines and CD34+ cells. Blood Cells Molec.& Diseases 23:69-87. **2. Guillaume-Gable *et al.* 1998.** Cationic phosphonolipids as nonviral gene transfer agents in the lung of mice. Hum Gene Ther 9:2309-2319.

STRUCTURE

LyoVec™ is a combination of the phosphonolipid DTCPTA and the neutral lipid DiPPE which structures are described below.



Di-tetradecylphosphoryl-N,N,N-trimethylmethanaminium chloride (DTCPTA)



1,2-Diphytanoyl-sn-Glycero-3-Phosphoethanolamine (DiPPE)

METHOD

LyoVec™ reconstitution

Add 2 ml of deionized sterile water per vial. Homogenize by vortexing gently for 30 sec. Place the vial at 4 °C for at least 30 min prior to initial transfection.

LyoVec™-GFP/SEAP reconstitution

1- Add 1 ml of deionized sterile water to the vial. Gently tap the vial to homogenize the complexes (do not vortex). Use directly or store at 4 °C.

LyoVec™ can be used for adherent cells as well as for cells grown in suspension. The table below shows indicative values for the volume of medium, the volume of LyoVec™/DNA complex and the number of cells for common transfection experiments. For your own experiments, you may need to optimize these values.

Culture format	96-well plate	24-well plate	12-well plate	6-well plate	100 mm dish
Medium per well/dish	200 µl	500 µl	1 ml	2 ml	8 ml
LyoVec™/DNA complex	10 µl	25 µl	50 µl	100 µl	400 µl
Cells per well/dish	1-5 x 10^4	1-2 x 10^5	2-4 x 10^5	5-10 x 10^5	3-5 x 10^6

Preparation of LyoVec™-DNA complexes

1- Bring LyoVec™ and LyoVec™-GFP/SEAP to room temperature and gently vortex to homogenize before use.
2- In a sterile 1.5 ml microfuge tube at room temperature, mix 1-3 µg plasmid DNA with 100 µl of LyoVec™. Mix gently.
Note: Prepare sufficient complex for the culture format you intend to use (see table above) using the same proportions: 1-3 µg plasmid DNA per 100 µl of LyoVec™.
3- Incubate at room temperature for at least 15 minutes to allow the formation of the complex.
4- Use the LyoVec™/DNA complexes for tranfection of cells or store them at 4 °C for further use.
Note: LyoVec™/DNA complexes remain fully active for transfection for at least 2 months when stored at 4 °C. Thus, preparation of large volumes of complexes can be made and reused repeatedly. Stored complexes must be resuspended by mixing before use and brought to room temperature.

Transfection of cells

1- Seed cells in culture medium containing serum in the dish or well as specified in the table above. Cells should be 50-80% confluent for best results. The transfection can be performed immediately after cell seeding.
Note: Cells can be added to dish or well before or after DNA/LyoVec™ complex without affecting transfection efficiency.
2- Add LyoVec™-DNA complexes directly to the medium at a 1/20 volume ratio (see table above). In a control dish or well, add 1/20 volume ratio of LyoVec™-GFP/SEAP complex . Swirl to distribute the complexes uniformly.
3- Incubate under appropriate conditions for 24-72 h to allow for gene expression then assay or apply antibiotic for selection.

For optimal transfection:

The ratio volume of cells/volume of complex is an important parameter for achieving optimal transfection, and may need to be optimized for your cells. In optimization experiments, test various cell/complex ratios by reducing or increasing the volume of complexes. Also note that LyoVec™ complexes work best at ratios for which a slight toxicity is observed.

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com