LyoVec™

A cationic lipid for transfecting mammalian cells

Catalog code: lyec-1, lyec-2, lyec-b

https://www.invivogen.com/transfection-reagents

For research use only

Version 23A17-NJ

PRODUCT INFORMATION

Contents:

LyoVec™ is supplied as a lyophilized lipid formulation to be reconstituted with water. It is compatible with all cell culture media. This product is available in 3 pack sizes:

- **lyec-1**: 5 small vials (125 µg per vial), sufficient to perform 200 reactions.
- **lyec-2**:10 small vials (125 µg per vial), sufficient to perform 400 reactions.
- **lyec-b**: bulk quantity upon request. Large vials (500 µg per vial), each sufficient to perform 160 reactions.

One reaction is a transfection of 2-4 x 10^5 cells/well in a 12-well plate.

Storage and stability:

- LyoVec™ is shipped at room temperature and should be stored at 4 °C. Lyophilized product is stable for 1 year when properly stored.
- After resuspension, store at 4 °C. Resuspended LyoVec™ is stable for 6 months when properly stored.

Quality control:

- Transfection efficiency of LyoVec™ has been confirmed in HEK293-derived cells.
- Cytotoxicity has been assessed in HEK293-derived cells.

DESCRIPTION

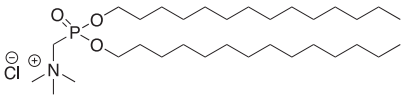
LyoVec™ is a proprietary cationic lipid belonging to the phosphonolipid family, originally described by Floch et *al.*, as an efficient transfection reagent both *in vitro* and *in vivo*[1,2]. The major constituent of LyoVec™ is the phosphonolipid DTCPTA, which is coupled to DiPPE, a neutral lipid that helps destabilize membrane bilayers and increases the *in vitro* transfection efficiency of LyoVec™. The positive charge of the phosphonolipid enables LyoVec™ to bind to plasmid DNA, where the structure promotes fusion with cellular membranes for efficient DNA delivery. LyoVec™ is designed as a transfection reagent for mammalian cells.

Additionally, LyoVec™ can be used as a nucleic acid complexing agent to facilitate the cellular entry of RNA or DNA-based oligonucleotides, such as RIG-I ligands (e.g. Poly(dA:dT)[3] and poly(I:C)[4]; see Related Products on the next page) and TLR7/8 ligands (e.g. ssPolyU[5]). This complexation step is crucial for inducing an effective response to the nucleic acids by intracellular pathogen recognition receptors.

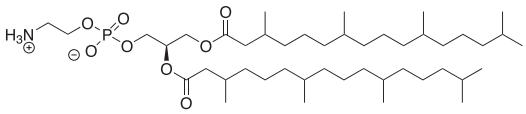
1. Floch *et al.* 1997. Cationic phosphonolipids as non viral vectors for DNA transfection in hematopoietic cell lines and CD34+ cells. Blood Cells Molec.& Diseases 23:69-87. **2. Guillaume-Gable *et al.* 1998.** Cationic phosphonolipids as nonviral gene transfer agents in the lung of mice. Hum Gene Ther 9:2309-19. **3. Xie M. *et al.*, 2016.** PKM2-dependent glycolysis promotes NLRP3 and AIM2 inflammasome activation. Nat Commun. 7:13280. **4. Järver P. *et al.*, 2018.** Single-Stranded Nucleic Acids Regulate TLR3/4/7 Activation through Interference with Clathrin-Mediated Endocytosis. Sci Rep. 8:15841. **5. Katashiba Y. *et al.*, 2011.** Interferon-α and interleukin-12 are induced, respectively, by double-stranded DNA and single-stranded RNA in human myeloid dendritic cells. Immunology. 132(2):165-73.

STRUCTURE

LyoVec™ is a combination of the phosphonolipid DTCPTA and the neutral lipid DiPPE (structures below).



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



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

APPLICATIONS

LyoVec™ can be used for adherent cells as well as cells grown in suspension. It has been validated for use as a:

- Cationic lipid-based transfection reagent
- Nucleic acid complexing agent

METHODS

Summary for preparing LyoVec™-nucleic acid complexes

01

Reconstitute lyophilized LyoVec™ with sterile H_2O and leave for 30 min at 15-25°C

02

Prepare the complexes at 10 µg/ml
- Add 1 µl of nucleic acid (1 mg/ml) to a 1.5 ml tube
- Add 100 µl of LyoVec™
- Mix gently by tapping the tube
- Incubate for 15 min -1 hour at 15-25°C
- Use immediately

LyoVec™ reconstitution

- Resuspend with deionized sterile water
  - Add 2 ml per small vial (cat. codes: **lyec-1** and **lyec-2**).
  - Add 8 ml per large vial (cat. code: **lyec-b)**.
- Homogenize by vortexing gently for 30 seconds.
- Leave vial at room temperature (15-25°C) for at least 30 minutes prior to initial use. Use immediately or store at 4°C.

The protocol for the preparation of LyoVec™-nucleic acid complexes and for the transfection of mammalian cells is given on the next page.

TECHNICAL SUPPORT
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Protocol for the preparation of LyoVec™-nucleic acid complexes

1. If stored at 4°C, bring LyoVec™ to room temperature and gently vortex to homogenize before use.
2. In a sterile 1.5 ml microfuge tube at room temperature, add 1 µl of nucleic acid (1 mg/ml). Add 100 µl of LyoVec™. Mix gently by tapping the tube.
3. Incubate at room temperature (15-25°C) for 15 minutes to 1 hour to allow for the formation of the complexes.
4. Use the LyoVec™-nucleic acid complexes immediately.

Note: Do not keep LyoVec™-nucleic acid complexes for more than 2 hours.

Transfection of cells

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.

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	5-10x 10^5	3-5 x 10^6

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 LyoVec™/DNA complex without affecting transfection efficiency.

2. Add LyoVec™-DNA complexes drop by drop to the medium at a 1/20 volume ratio (see table above). Swirl to distribute the complexes uniformly.
3. Incubate under appropriate conditions for 24-72 hours 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.

RELATED PRODUCTS

Product	Description	Cat. Code
Cytosolic DNA sensors (CDSs) ligands		
G3-YSD	Y-form DNA	tlrl-ydna
HSV-60 Naked	Viral DNA motif	tlrl-hsv60n
VACV-70 Naked	Viral DNA motif	tlrl-vav70n
Endosomal TLR ligands		
ssPoly(U) Naked	TLR7/TLR8 ligand	tlrl-sspu
RIG-I ligands		
3p-hpRNA	5' triphosphate hairpin RNA	tlrl-hprna
5'-ppp-dsRNA	5' triphosphate dsRNA	tlrl-3prna
Poly(dA:dT)	Synthetic analog of B-DNA	tlrl-patn
Poly(I:C) HMW	Synthetic dsRNA polymer	tlrl-pic
Expression plasmids		
pUNO1-mcs	Cloning plasmid	puno1-mcs
pMONO-zeo-mcs	For a single gene	pmonoz-mcs
pDUO-mcs	For two genes	pduo-mcs
Expression plasmids - to generate tagged proteins		
pSELECT-NGFP-zeo	GFP-N-terminal tag	psetz-ngfp
pSELECT-NHA-zeo	HA-N-terminal tag	psetz-nha
pSELECT-NHis-zeo	HIS-N-terminal tag	psetz-nhis
pSELECT-NLucia-zeo	Lucia-N-terminal tag	psetz-nlucia
Expression plasmids - for antibody production		
pFUSE-CHIg-hG1	Heavy chain, human IgG1	pfuse-hchg1
pFUSE2-CLIg-hK	Light chain, human Kappa	pfuse2-hclk
pTRIOZ-hIgG1	Heavy & light chains	ptrioz-higg1

For a complete list of our cloning and expression vectors, visit https://www.invivogen.com/vectors

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