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 - Absence of inflammatory response using *in vivo*-jetPEI™

Meet the Polyplus team at the following meetings:

- ▶ **20-24 October, 2008**
Discovery on Target
Boston, MA, USA, the 6th Annual RNAi for Screening meeting - Booth 28
- ▶ **13-16 November, 2008**
ESGCT 2008
Bruges, Belgium, the 16th Annual Congress of the European Society of Gene and Cell Therapy - Booth 1.4
- ▶ **13-17 December, 2008**
ASCB 2008
San Francisco, CA, USA, the 48th Annual Meeting of the American Society for Cell Biology - Booth 1735

Transfection for Recombinant Protein and Virus Production

Transfection and biomolecule delivery to mammalian cells represents a powerful technique to produce a range of recombinant proteins such as human therapeutic molecules including proteins, monoclonal antibodies and vaccines. Protein expression is routinely performed at a small scale for research purposes and at larger scale to manufacture therapeutic proteins and recombinant viruses such as lentiviruses. Recently, most efforts have focused on transient transfection since it is a time-saving procedure compared to the generation of stable cell lines. Whatever the goal, the use of robust transfection reagents is required to ensure efficient and sustained protein expression levels. Furthermore batch to batch reproducibility needs to be guaranteed. This is better achieved using high quality grade synthetic and chemically-defined reagents.

For everyday protein production in mammalian cells, robust synthetic reagents, such as jetPEI™ are recommended mainly due to their efficiency in the presence of serum which keeps the cells in a healthy physiological state. This in turn increases protein and virus expression in routine media such as DMEM. Recently, Brun *et al.*, (2008) used this reagent to produce HIV-1 viral stocks in 293T cells for an interesting structure/function study. They showed that some specific mutations in the HIV capsid lead to assembly defects and instability of the core.



Typical example of a flask used for virus or protein production.

This defect is probably due to the endocytic pathway used. Interestingly, these mutations also affect reverse transcription and nuclear import leading to reduced viral infectivity.

For applications requiring cells to grow in suspension or in synthetic media, very gentle transfection reagents are needed to avoid excessive strain on the cellular machinery. For example, Fecturin™-mediated DNA transfection is milder on the cells allowing a faster recovery from transfection leading to sustained robust protein expression for routine viral stock production, recombinant protein expression and generation of stable cell lines (Barile *et al.*, 2008) for preclinical and clinical applications.



One liter of transfection reagent.

The demand for GMP-grade or GMP compatible reagents is currently increasing steadily in bioproduction laboratories. For example, high quality transfection reagents are required for the manufacturing of therapeutic viruses used in the clinics, where production protocols have become more stringent. Polyplus-transfection reagents are perfectly suited for such technical and regulatory constraints. With a department dedicated to high quality grade reagents, Polyplus-transfection provides prime and personalized service including scientific support to suit the pharmaceutical industry requirements.

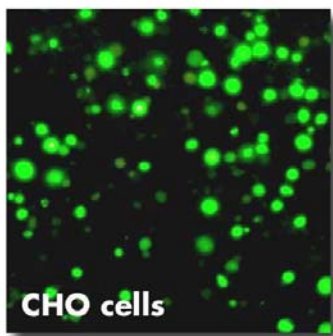
Barile *et al.*, *Organic and Biomolecular Chemistry* (2008), **6**, 1756-62

Brun *et al.*, *Retrovirology* (2008), **5**:57

Protein Production with Fecturin™

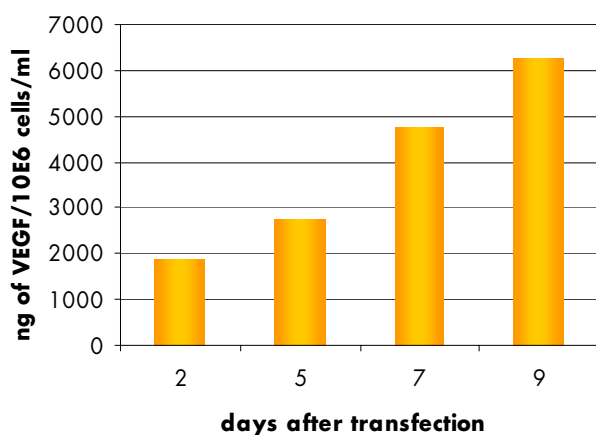
Fecturin™ is particularly well-suited for DNA transfection of high density suspension mammalian cell culture leading to high levels of protein production.

- ▶ Efficient in synthetic and standard media
- ▶ Adapted to cells grown in suspension
- ▶ Very gentle to cells: high protein production level
- ▶ Guaranteed high grade and animal free, thus suitable for drug biomanufacturing
- ▶ Batch to batch reproducibility
- ▶ Sustained protein production over several days
- ▶ Ideal for large scale production
- ▶ Virus bioproduction

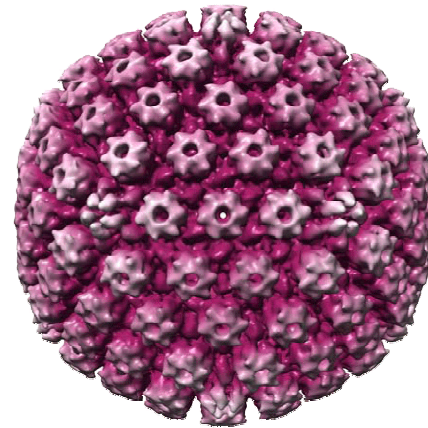


Transfection of CHO cells using Fecturin™. Cells were grown in a commercially available synthetic media, transfected with pCMV-EGFP plasmid following the standard Fecturin™ protocol and visualized by fluorescent microscopy one day post-transfection.

Cumulative VEGF production in CHO cells in suspension



CHO cells were grown in a synthetic medium at 37°C, 8% CO₂ with constant shaking. Cells were transfected with 2 µg of plasmid DNA per ml of cell culture medium and 3 µl of Fecturin™ per µg of DNA. VEGF protein production was quantified by ELISA.

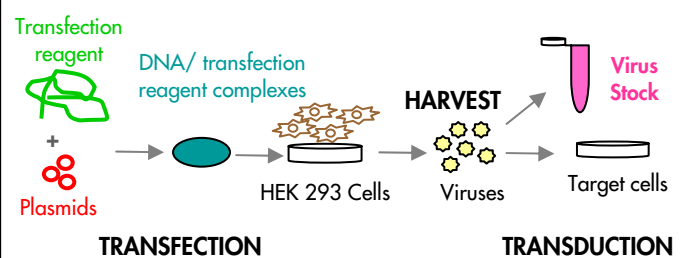


Virus Production with Fecturin™

Fecturin™ is suitable for routine virus production in adherent cell lines grown in standard media in the presence of serum.

- ▶ Efficient in standard medium such as DMEM
- ▶ Widely used for adherent cells
- ▶ Adapted to routine applications
- ▶ Easy protocol:
 - No need to change medium
 - Compatible with serum and antibiotics
- ▶ Over 70% transfection efficiency in HEK 293 cells
- ▶ High viral titers:
 - >10⁷ TU/ml for lentivirus
 - >10⁹ TU/ml for AAV
- ▶ Batch to batch reproducibility

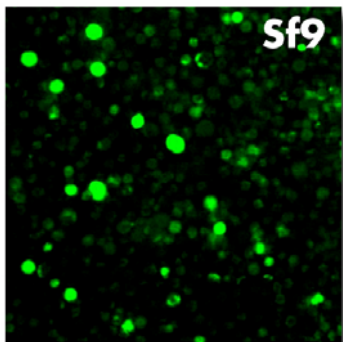
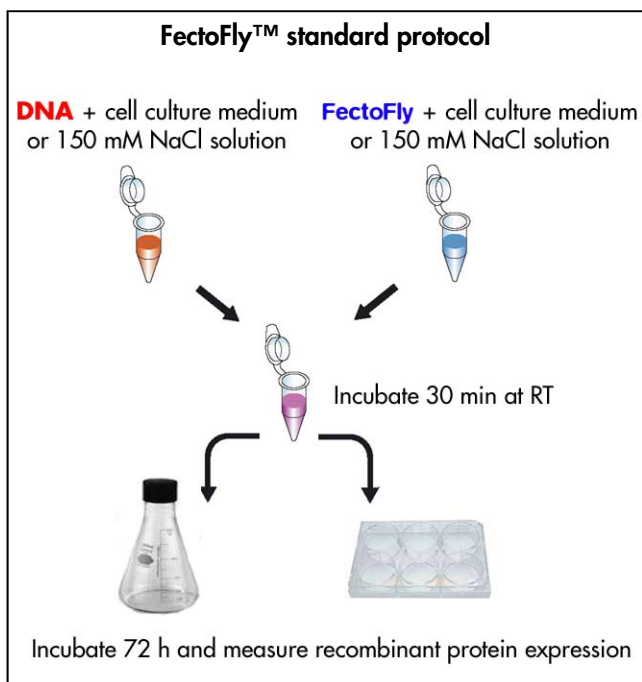
Production of recombinant viruses



Baculovirus production in insect cells with FectoFly™

FectoFly™ is a set of DNA transfection reagents dedicated to insect cells for high level production of baculovirus and recombinant proteins.

- ▶ Dedicated insect cell reagents
- ▶ Transfection kit suitable for a wide variety of insect cells
 - Sf9, Sf21, Tn5
- ▶ High protein expression and baculovirus production
- ▶ Guaranteed free of materials from animal origin
- ▶ Adapted to both adherent and suspension cells

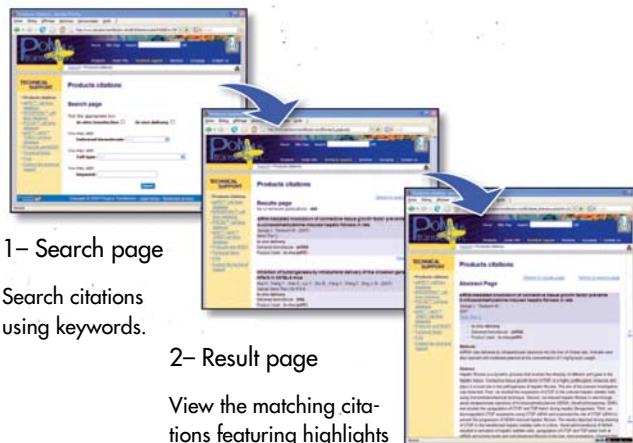


GFP expression in Sf9 cells grown in suspension 72 h after transfection using FectoFly™ I.

The online product citation database on www.polyplus-transfection.com

A helpful tool for your literature searches

Under the Technical Support Heading, you will find a powerful tool enabling you to search published citations featuring Polyplus-transfection reagents.



1– Search page

Search citations using keywords.

2– Result page

View the matching citations featuring highlights of products used as well as cell types and methods.

3– Abstract page

View the abstract and use the link to go to PubMed.

For additional information, please contact our technical support service:

support@polyplus-transfection.com

Product	Cat N°	Reagent	Amount of transfected DNA
Fecturin™	109-TEST	0.3 ml	0.1 to 0.3 mg
	109-001	1 ml	0.3 to 1 mg
	109-010	10 ml	3 to 10 mg

Product	Cat N°	Reagent	Amount of NaCl sol.
jetPEI™	101-10	1 ml	-
	101-10N	1 ml	50 ml
	101-40	4 x 1 ml	-
	101-40N	4 x 1 ml	4 x 50 ml

Product	Cat N°	Reagent	Amount of NaCl sol.
FectoFly™ trial kit	113-02N	2 x 0.1 ml	10 ml
FectoFly™ I	111-10	1 ml	-
FectoFly™ II	112-10N	1 ml	50 ml

Bulk sizes available upon request.

HOT Publications: *in vivo* DNA & siRNA delivery

Intratumoral DNA Delivery

Jedy, G., *et al* (2008). *Cancer Gene Ther* (epub ahead of print)

The authors used *in vivo*-jetPEI™ for intratumoral delivery of plasmid DNA encoding for the superantigen *Staphylococcus enterotoxin A* (SEA). SEA gene delivery, coupled with epinephrine infusion, stimulated an antitumor immune response against subcutaneous melanoma cells, leading to tumor growth inhibition.

Systemic siRNA Delivery

Yang, D. Y., *et al* (2008). *Nature* (epub ahead of print)

Yang *et al* demonstrated that TMEM16A presents the characteristics of calcium-activated chloride channels (CaCC), both *in vitro* and *in vivo*. Using siRNA complexed with *in vivo*-jetPEI™ and delivered systematically, they showed that TMEM16A downregulation leads to reduction of saliva production, a typical phenotype of CaCC depletion.

Lively, T. N., *et al* (2008). *J Allergy Clin Immunol* 121, 88-94

siRNAs modified to reduce dsRNA-induced immune response were delivered systemically using *in vivo*-jetPEI™ with the goal of treating allergen-induced airway hyperresponsiveness in sensitized and allergen challenged mice.



The transfection dedicated company

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Let us know about your Publications



If you have published a paper with Polyplus reagents, we would love to hear from you. Please send us a copy of your work to support@polyplus-transfection.com and we will send you a complimentary laser pointer!

Latest publications from our R&D team

Intracellular antibody delivery

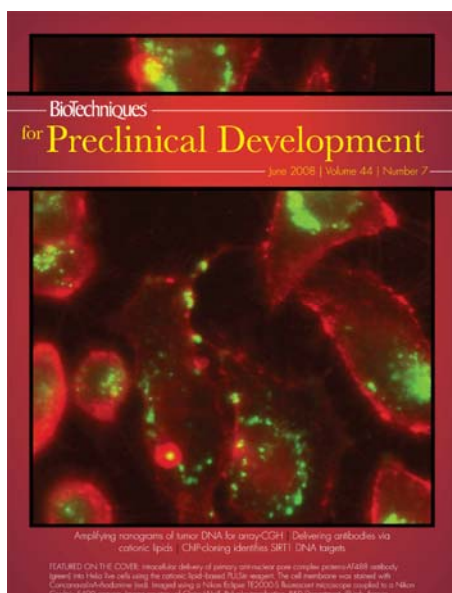
Weill, C. O., *et al* (2008). Cationic lipid-mediated intracellular delivery of antibodies into live cells. *BioTechniques* 44, Pvi-Pxi.7

Our R&D team demonstrated the efficient intracellular release of delivered labeled antibodies upon delivery with PULSin™ and compared their results into live cells with immunocytochemistry experiments in fixed cells.

Absence of inflammatory response using *in vivo*-jetPEI™

Bonnet, ME., *et al* (2008). Systemic Delivery of DNA or siRNA Mediated by Linear Polyethylenimine (L-PEI) Does Not Induce an Inflammatory Response. *Pharm Res* (ahead of print)

We analysed the inflammatory response following *in vivo*-jetPEI™ mediated DNA, siRNA or sticky siRNA delivery by systemic injection. No major production of pro-inflammatory cytokines or hepatic enzymes was observed. Our data highlight that *in vivo*-jetPEI™ is a delivery reagent of choice for nucleic acid therapeutics.



TRADEMARKS

Polyplus-transfection, FectoFly, Fecturin, INTERFERin, jetPEI and PULSin are registered trademarks of Polyplus-transfection SA.

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LICENCING & PATENT INFORMATION

Polyplus-transfection owns the exclusive worldwide license (#133139, Registre National des Brevets, 14/05/2003) for the transfection of nucleic acids using polyethylenimine and derivatives (Boussif *et al.*, 1995).

Fecturin™ is under the patent right N° WO 2006/087243 from Polyplus-transfection.