Invitrogen Transfecion Reagents

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Transfection Overview

What is transfection?

Technology to introduce nucleic acids or nucleotides into eukaryotic cells

What do researchers do with it?

- Study regulation of gene expression
- Explore functional pathways in living cells
- Investigate gene silencing by RNAi or antisense oligos
- Produce recombinant proteins, antibodies
- Different customers by application and throughput
  - Applications: specific cell-types/multiple cell-types, RNAi knockdown, DNA delivery, large-scale protein/AB production

What methods are used for delivering nucleic acids into cells?

- Cationic lipids (most common method)
- Polyethyleneimines, activated dendrimers, lipid-protein polymer
- Chemical methods like calcium phosphate, DEAE-dextran
- Electroporation (amaxa, Bio-Rad, BTX)
- Viral methods (adenoviral, retroviral, lentiviral)
- Protein-mediated delivery (viral peptides)

Invitrogen Transfection Reagents Portfolio

1988 1990 1995 1996 1999 2003 2005 2006

Lipofectin®

Cellfectin®

Lipofectamine™ 2000

DMRIE-C

Lipofectamine™

PLUS™

293fectin™

FreeStyle™ Expression System

Oligofectamine™

New Products in 2006

Lipofectamine™ RNAiMAX

Lipofectamine™ LTX

FreeStyle™ MAX

Lipofectamine™ 2000 CD

Optifect™

Lipofectamine™ 2000

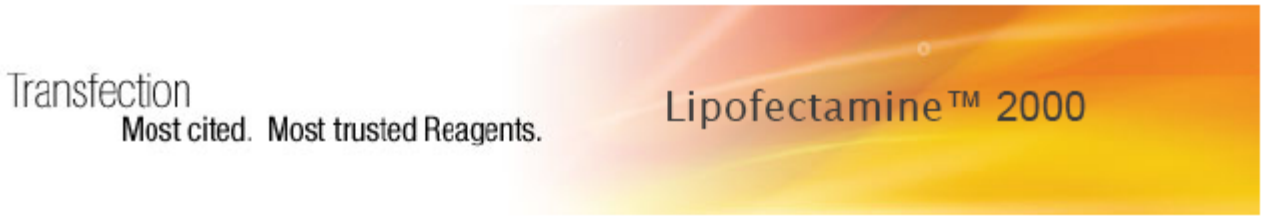
- Most Cited, Most Trusted -

Lipofectamine™ 2000 Features and Advantages

High Expression, High Efficiency, High Knockdown
Many popular cell lines transfected at >90% efficiency

Why is high expression & efficiency important?

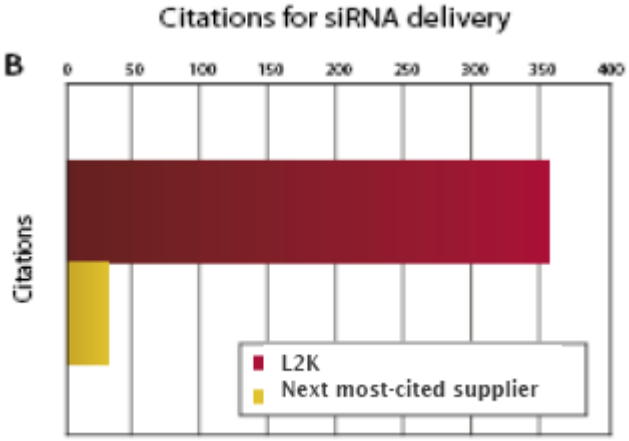
- More cells express gene or reporter gene of interest – more cells respond to assay, more efficient data analysis
- Optimized protocols mean fewer experimental repeats – faster results
- Less reagent to achieve optimal performance – cost savings per assay
- Higher protein yields – more cells are expressing, faster results



General purpose reagent for both DNA and RNAi delivery

Lipofectamine™ 2000 – Best reagent

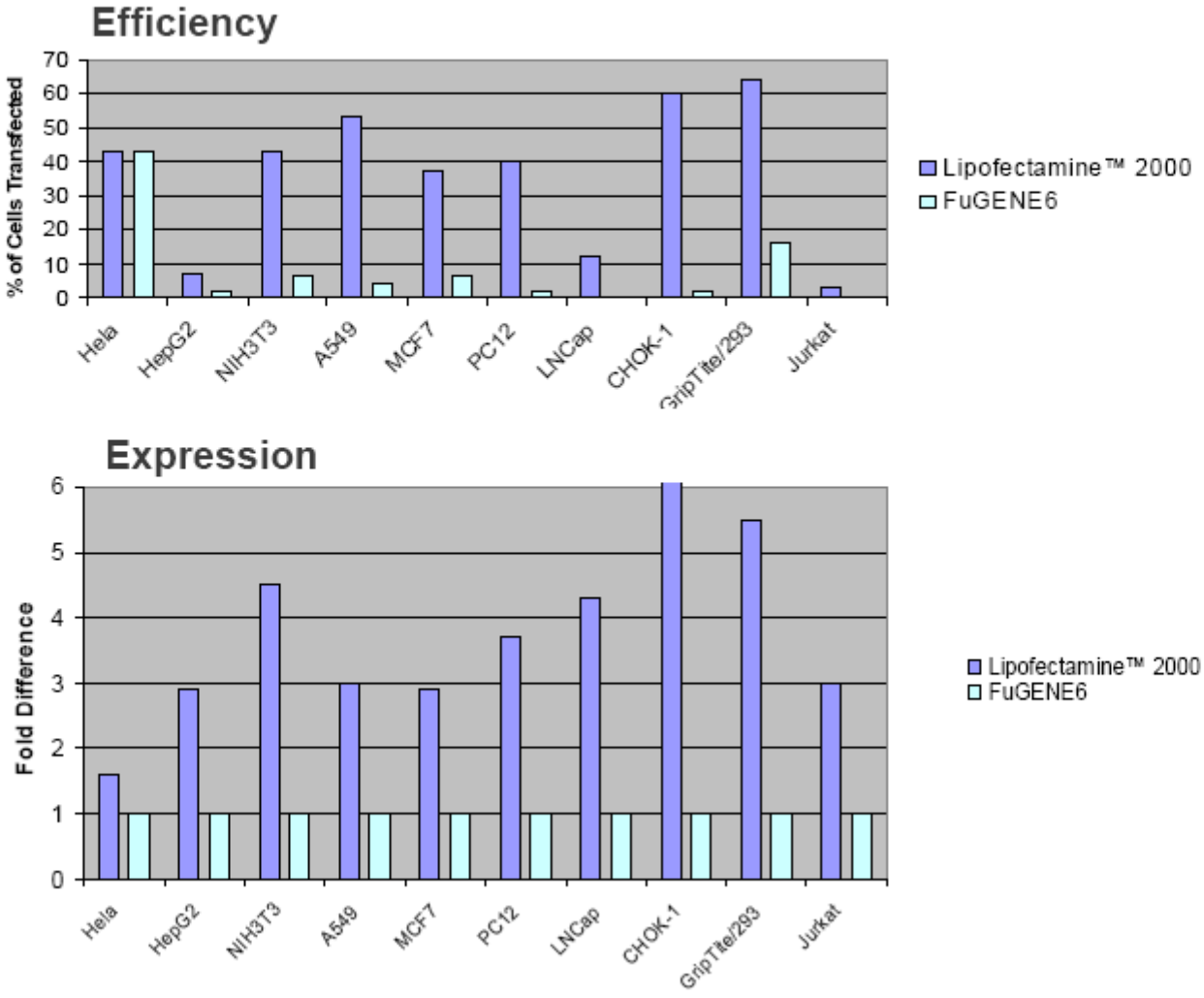
- for delivery of both DNA and RNA
- Nucleic acid-Lipofectamine 2000 complexes can be added directly to cells in culture medium, in the presence or absence of serum
- not necessary to remove complexes or change/add medium after transfection
- most cited transfection reagent



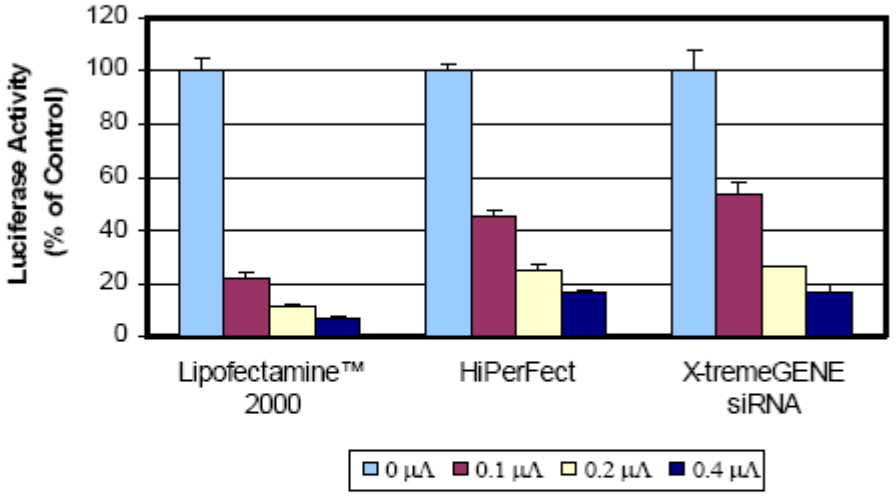
Lipofectamine™ 2000 – high efficiency

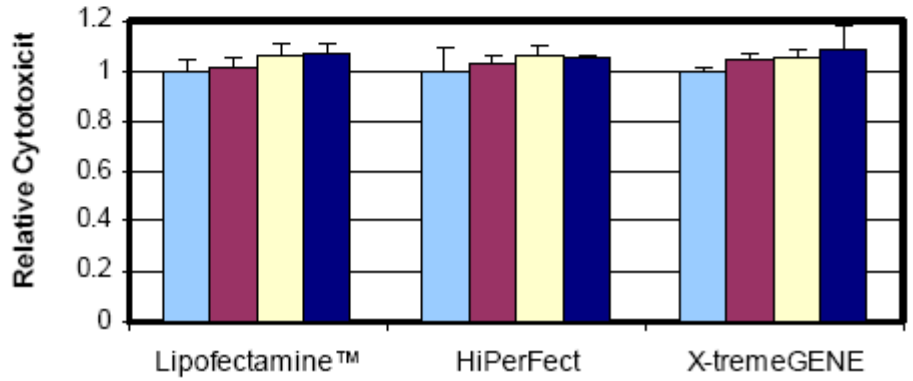
Cell Line	Cell Type	Transfection efficiency (%)
HEK-293	Human kidney	99
CHO	Hamster ovary, adherent	96
COS-7L	Monkey kidney	99
BE(2)C	Human neuroblastoma	77
SKBR3	Human breast cancer	49
MDCK	Dog kidney	43
HT1080	Human fibrosarcoma	81
Fibroblasts	Human primary	48
HeLa	Human cervical carcinoma	94
CV-1	Monkey kidney	70
Vero	Monkey kidney	86
PC12	Rat pheochromocytoma	85
Murine ES	Mouse embryonic stem cells	75
Rat Hepatocytes	Primary liver	50
E18 Cortical Neurons	Rat primary	25
E18 Hippocampal Neurons	Rat primary	30

Plasmid Delivery - L2K vs. Roche FuGENE® 6



Lipofectamine™ 2000 – RNAi application



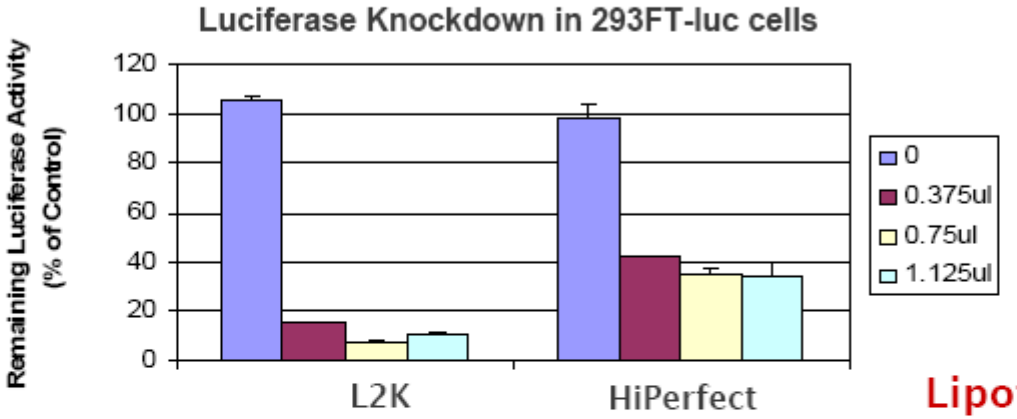


Lipofectamine™ 2000

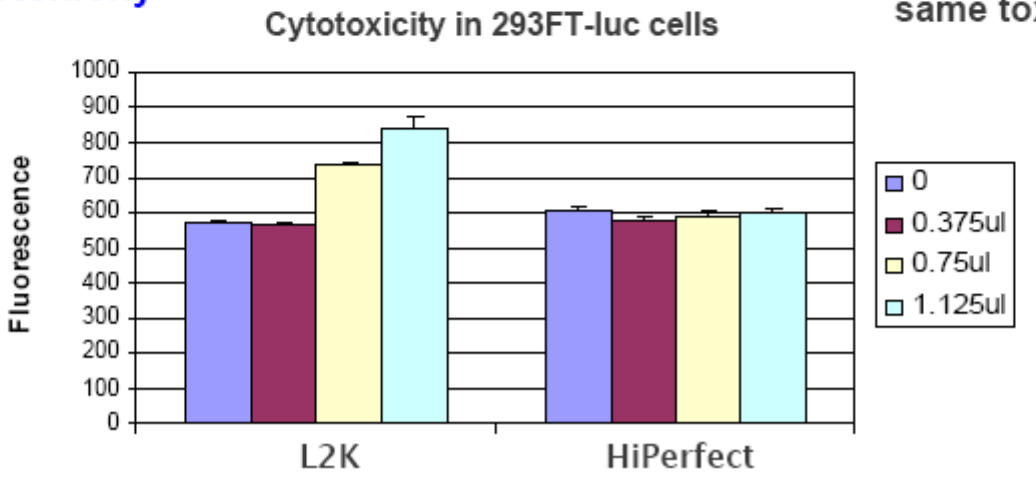
- lower siRNA concentraion
- higher knock down efficiency

RNAi Delivery - L2K vs. Qiagen HiPerFect

Gene Silencing



Cytotoxicity



Lipofectamine™ 2000

higher knock down efficiency at same toxicity level

Lipofectamine™ LTX

- Low Toxicity -

Lipofectamine™ LTX Features and Advantages

Lipofectamine™ LTX: plasmid DNA-specific transfection reagent that provides maximum gene expression and lowest cytotoxicity

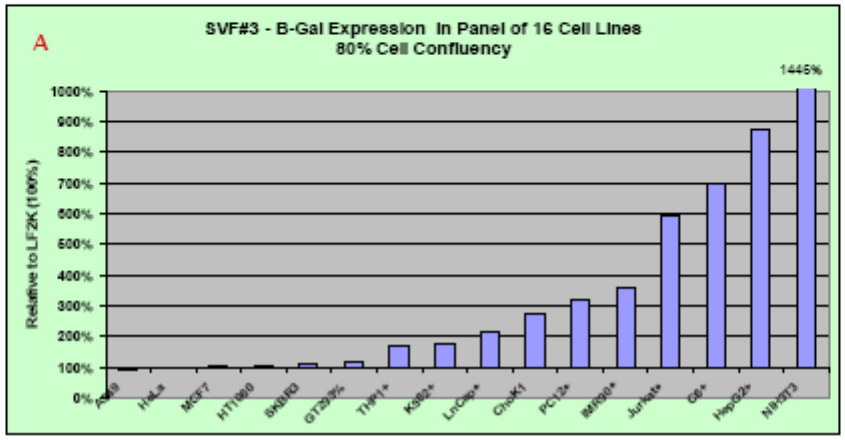
Key Features

- Higher transfection efficiency and significantly lower toxicity levels **(>90% cell viability)** for a wide range of cell lines
- Significantly improved transfection performance in **primary and disease-relevant cell lines (primary neuronal cells)**
- Optimized protocols for over 20 different cell types are available now, less time for evaluation and optimization

Lipofectamine™ LTX - Efficiency and Toxicity

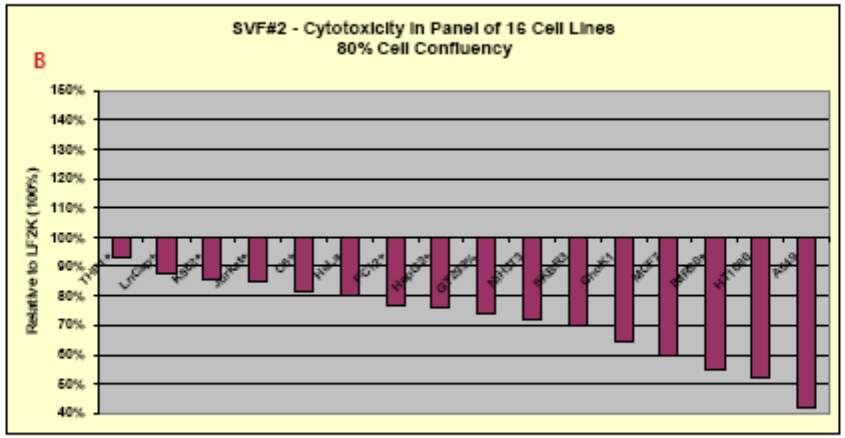
1	A549
2	C6
3	ChoK1
4	GT293
5	HeLa
6	HepG2
7	HT1080
8	IMR90
9	Jurkat
10	K562
11	LnCap
12	MCF7
13	NIH3T3
14	PC12
15	SKBR3
16	THP1

113,271 Pub Med Hits



15/16 equivalent or higher expression compared to Lipofectamine™ 2000

Average Increase 200%

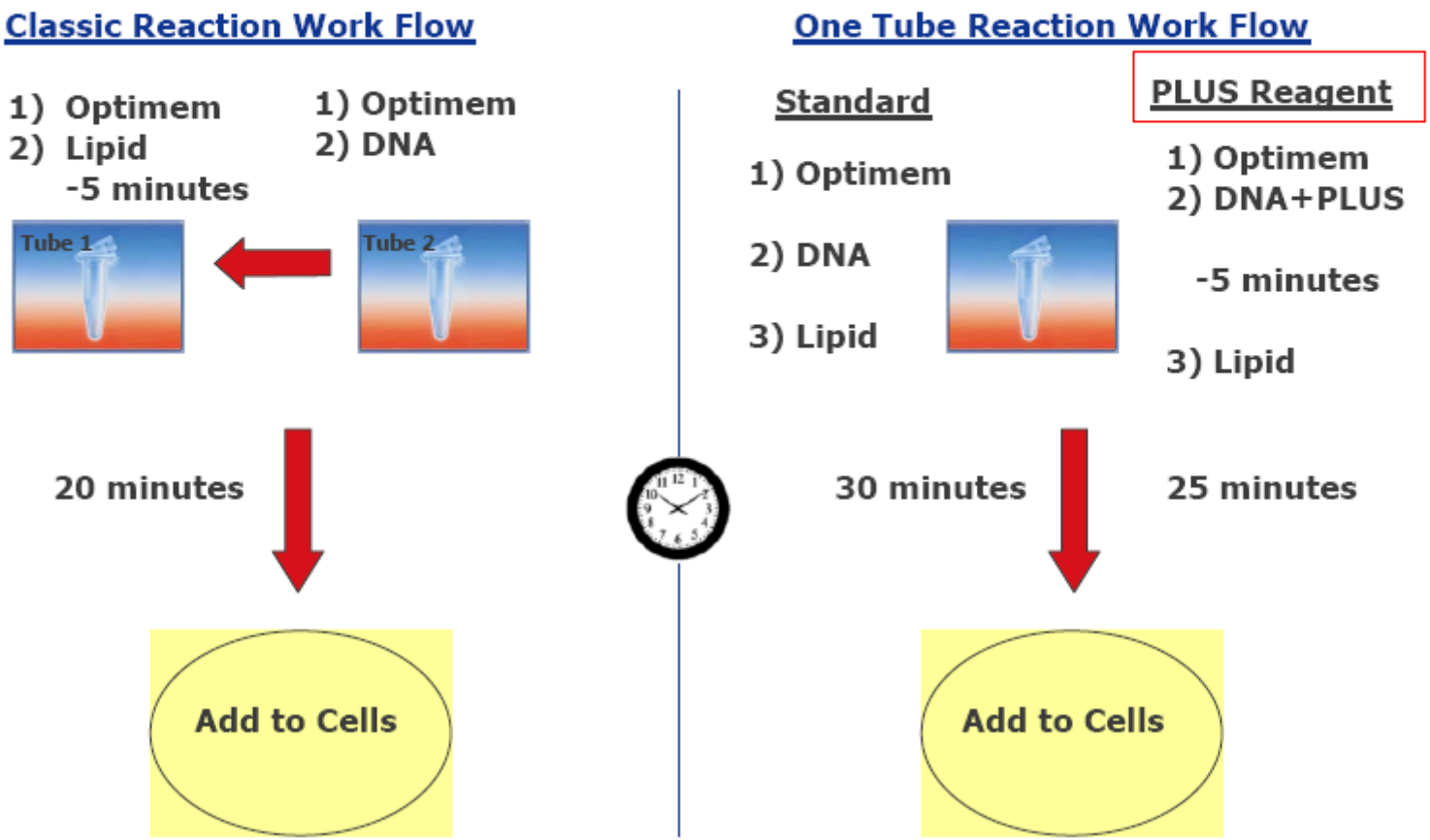


15/16 have 10% or more decreased cytotoxicity compared to Lipofectamine™ 2000

Average reduction 27%

* High and Low Values Omitted

Lipofectamine™ LTX - Basic Use Protocol Improvement



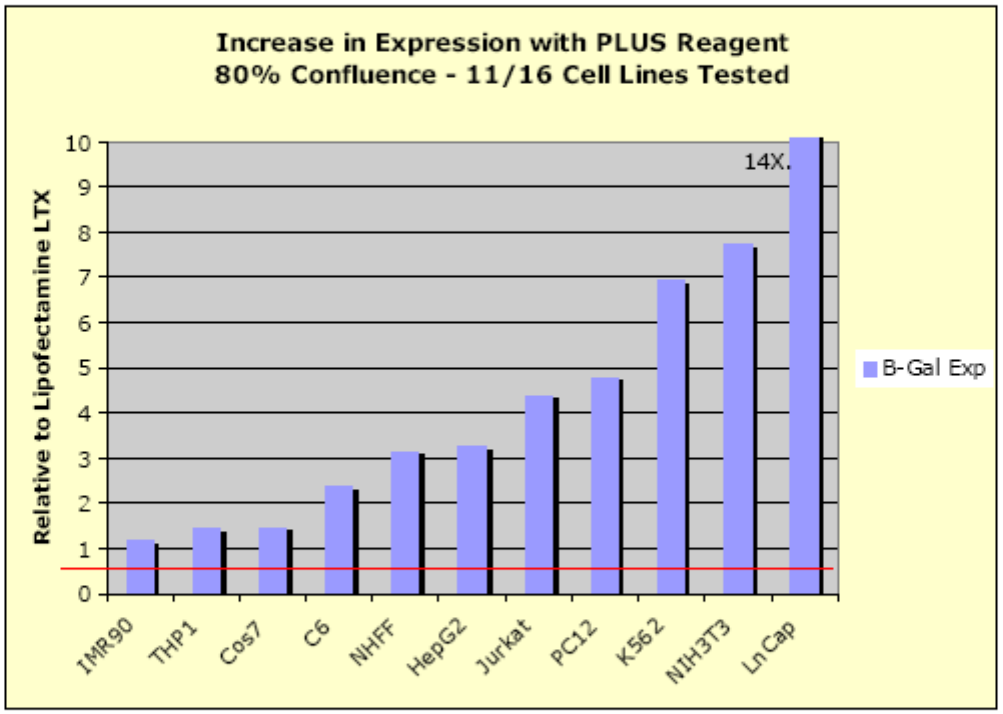
Plus Reagent

- proprietary reagent for pre-complexing DNA
- enhances cationic lipid-mediated transfection of DNA
- may be inhibited by some serum-free media
  (such as CD293, 293 SFM II and VP-SFM)

Plus Reagent Enhancement

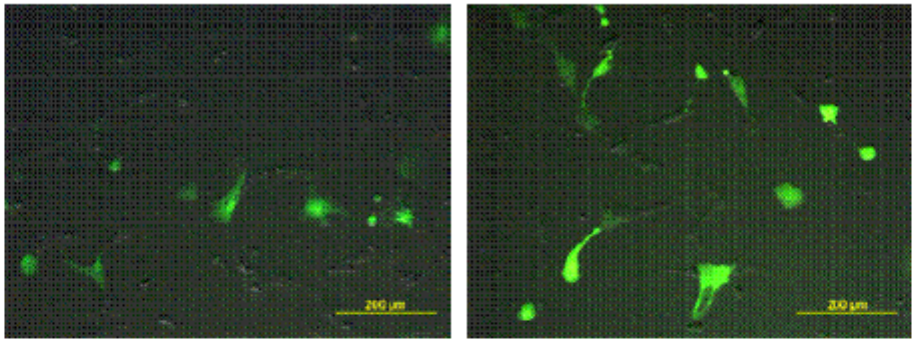
Plus™ Reagent

Pre-complexation of DNA with Plus™ Reagent, 5-15 minutes



- Significant increase in expression vs Lipofectamine™ LTX alone
- No change in cytotoxicity

Transfection of Neural Progenitor Cells with LTX

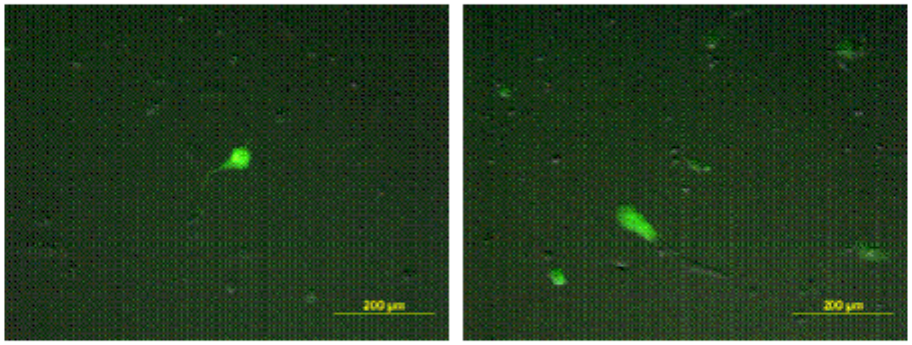


A

10 µl of LTX

B

10 µl of LTX +
2.5 µl of Plus™ Reagent

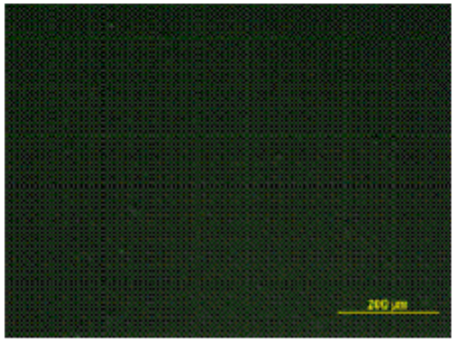


C

15 µl of LTX +
2.5 µl of Plus™
Reagent

D

10 µl of LF 2000

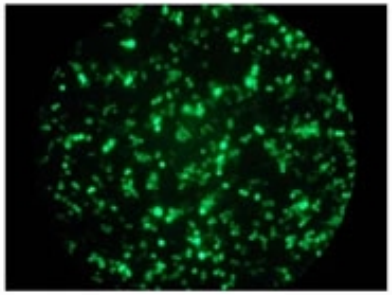


E

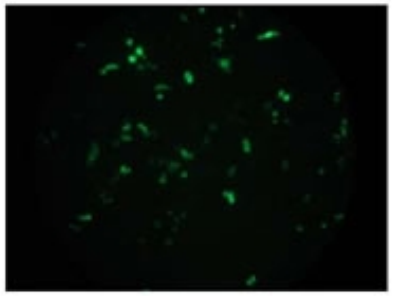
Untransfected cells

Courtesy of
Dr. Beverly Davidson,
Univ of Iowa

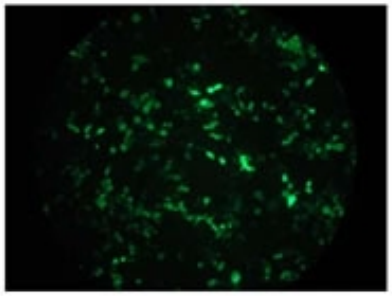
Transfection of RAW267.3 Macrophage Cells with LTX



Lipofectamine™ LTX



FuGENE® 6



FuGENE® HD

Each reagent was used to transfect RAW267.3 macrophage cells in a 96-well format, and GFP analyzed 48 hr posttransfection. Lipofectamine™ LTX Reagent, in the presence of PLUS™ Reagent, provided higher transfection efficiency and GFP expression than the FuGENE® 6 and FuGENE® HD reagents. Data courtesy of the Ron Evans laboratory, The Salk Institute.

Lipofectamine™ LTX - Improved Transfection Protocol

Protocol	Lipofectamine™ 2000	Lipofectamine™ LTX
Pre-dilution of Lipid	Essential	Not Required (see 1 Tube Protocol)
Dilution Media	Optimem, lowered effectiveness in OptiPro, RPMI and DMEM.	Optimem Recommended, OptiPro, RMPI and DMEM also work
Lipid Dilution Stability	0.5-5 minutes	0.5-5 minutes
Complexation	20 minutes	30 minutes
Complex Stability	Begins to diminish after 30min	Stable out to 240min
Serum Inhibition	No effect Media containing serum	No effect Media containing serum
Antibiotic Toxicity	Not Recommended	OK with some cell types
Post-Transfection Expression measured 24, 28, and 72 hours post transfection	Expression measured 24, 28, and 72 hours post transfection	Expression 72 hours post transfection is higher in 2/3 cell lines
Stable Cell Lines	Selection with Blasticidin complete in 7 days.	Selection with Blasticidin complete in 7 days
Scalability 96-6 well	Linear scale	Linear scale
Reverse Transfection	Protocol Provided	Protocol Provided

Lipofectamine™ RNAiMAX

- siRNA Transfection -

RNAiMAX Features and Advantages

Lipofectamine™ RNAiMAX: RNAi-specific transfection reagent that offers the highest transfection efficiencies, highest knockdown levels on the widest variety of cell types for siRNA experiments

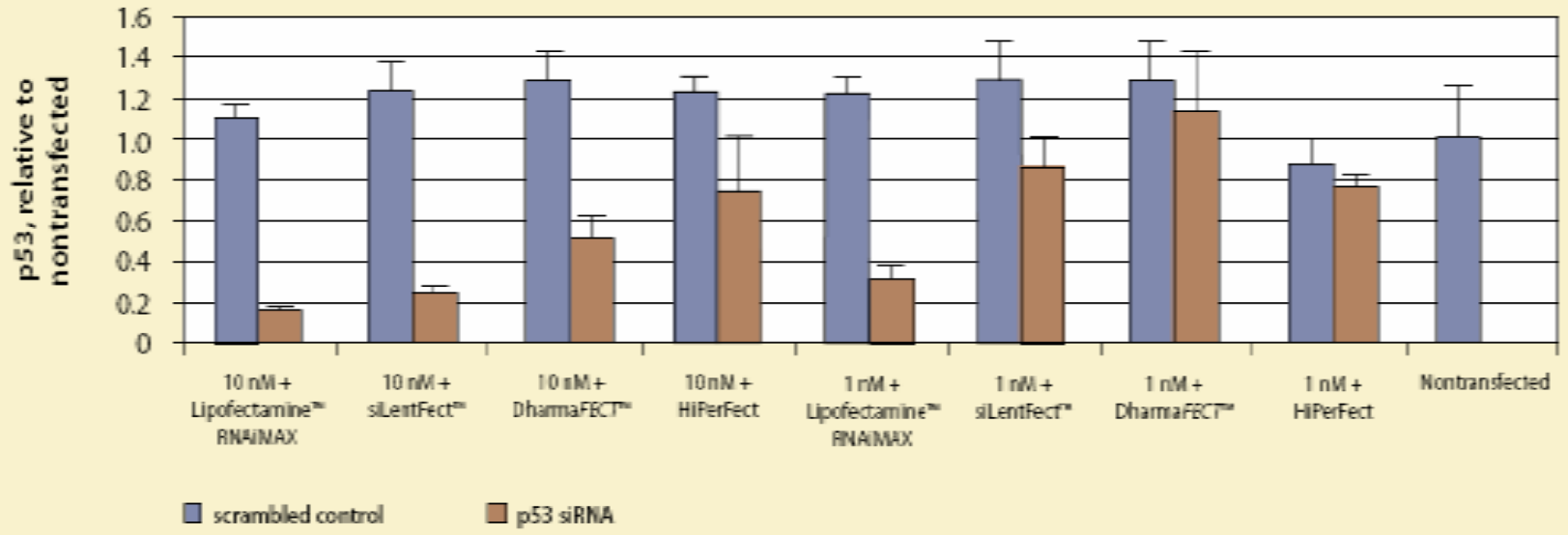
Key Features

- Superior transfection efficiency requiring lower RNAi concentrations leading to more effective gene knockdown with **minimal non-specific effects**
- Easy optimization from to minimal cytotoxicity across a 10-fold concentration range of transfection reagent
- Compatible with broad range of cell types providing the most flexible approach to all of your gene silencing experiments

Stealth™ RNAi Technology

Superior Transfection at Low siRNA Concentrations

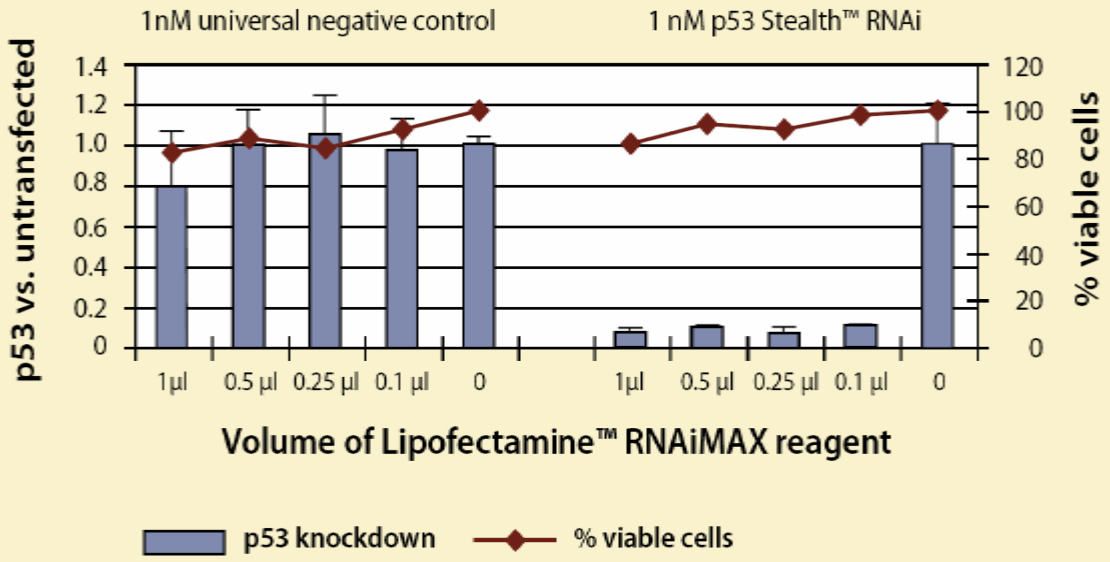
Figure 1—Superior knockdown with Lipofectamine™ RNAiMAX Transfection Reagent compared with competing siRNA transfection reagents



At both 10 nM and 1 nM p53 siRNA, Lipofectamine™ RNAiMAX Transfection Reagent provides more effective knockdown than other RNAi reagents, including siLentFect™ (Bio-Rad), DharmaFECT™ (Dharmacon), and HiPerFect (Qiagen) reagents.

Low Cytotoxicity Profile for Easy Optimization

Figure 2—Optimal knockdown and minimal cytotoxicity in A549 cells transfected with Lipofectamine™ RNAiMAX Transfection Reagent



A range of 0.1 µl to 1.0 µl of Lipofectamine™ RNAiMAX was used in the experiment resulting in efficient knockdown of the p53 gene expression level while maintaining excellent cell viability in transfected cells.

Optimized Protocols Available for Many Cell Lines

Cell line/type	Cell type
MDA-MB-435	Breast cancer
HeLa	Cervical carcinoma
HT1080	Human fibrosarcoma
A549	Lung carcinoma
HepG2	Liver carcinoma
MCF7	Breast cancer
SK-N-SH	Neuroblastoma
Mesenchymal stem cells	Bone marrow
HCT116	Colon carcinoma

DMRIE-C

- Suspension Cells / RNA Transfection -

DMRIE-C (1,2-DiMyristyl Rosenthal Inhibitor Ether) : suspension cells

- 1:1 liposome formulation of cationic lipid DMRIE and cholesterol in water
- Transfection of DNA and RNA into eukaryotic cells
- Particularly effective for suspension cells (e.g. Jurkat) and other lymphoid-derived cell lines

FreeStyle™ MAX

- Protein Production -

FreeStyle™ MAX for Protein Production

Description

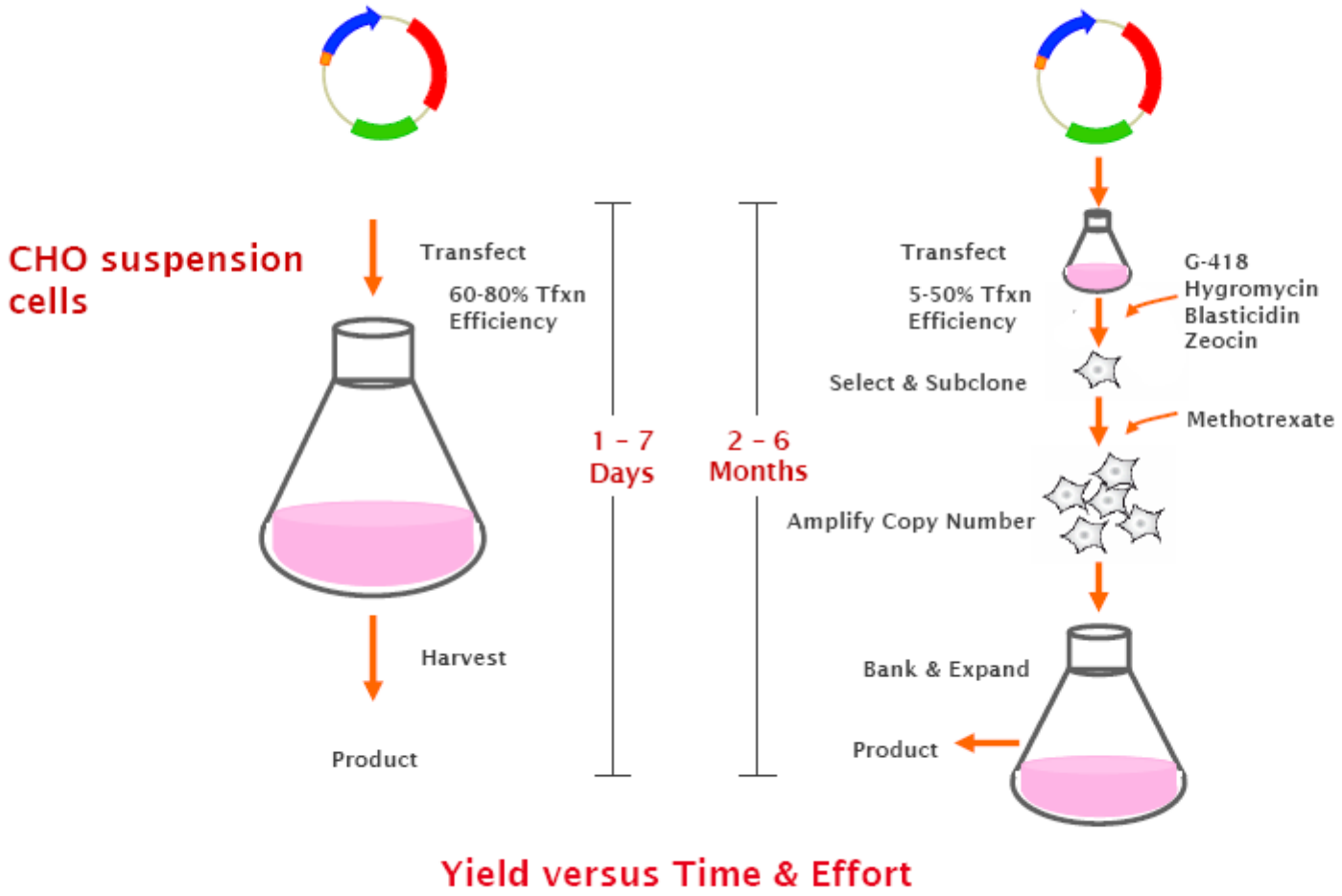
FreeStyle™ MAX System is a complete optimized system for plasmid transient transfections in suspension CHO and HEK-293 cultures with **high protein yield**, **high transfection efficiency**, completely **animal-origin free** components, and **scalability** to accommodate a wide range of protein production applications

Key features

- Easy, rapid protocol, transiently transfected cells in suspension culture
- Functional proteins produced with full post-translation modifications, similar to proteins used in downstream therapeutic manufacturing
- Simplified protein purification of secreted proteins through use of the FreeStyle serum-free medium
- Convenient, reproducible scale-up from 30 ml to 10 liters of culture
- Available in complete kit, individual reagents, services

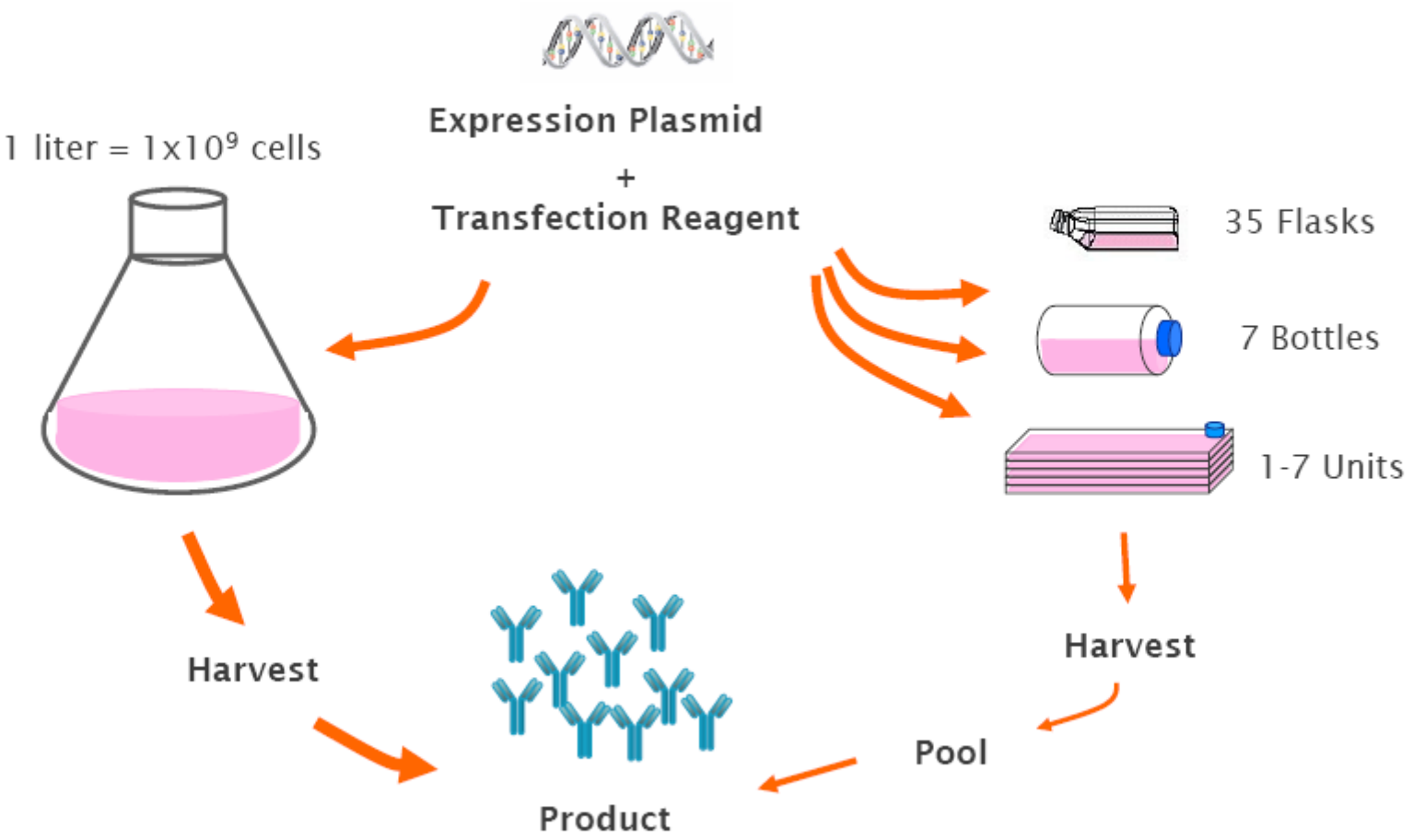
Generate milligram quantities of proteins in days...saves time

Transient Transfection vs. Stable Cell Generation



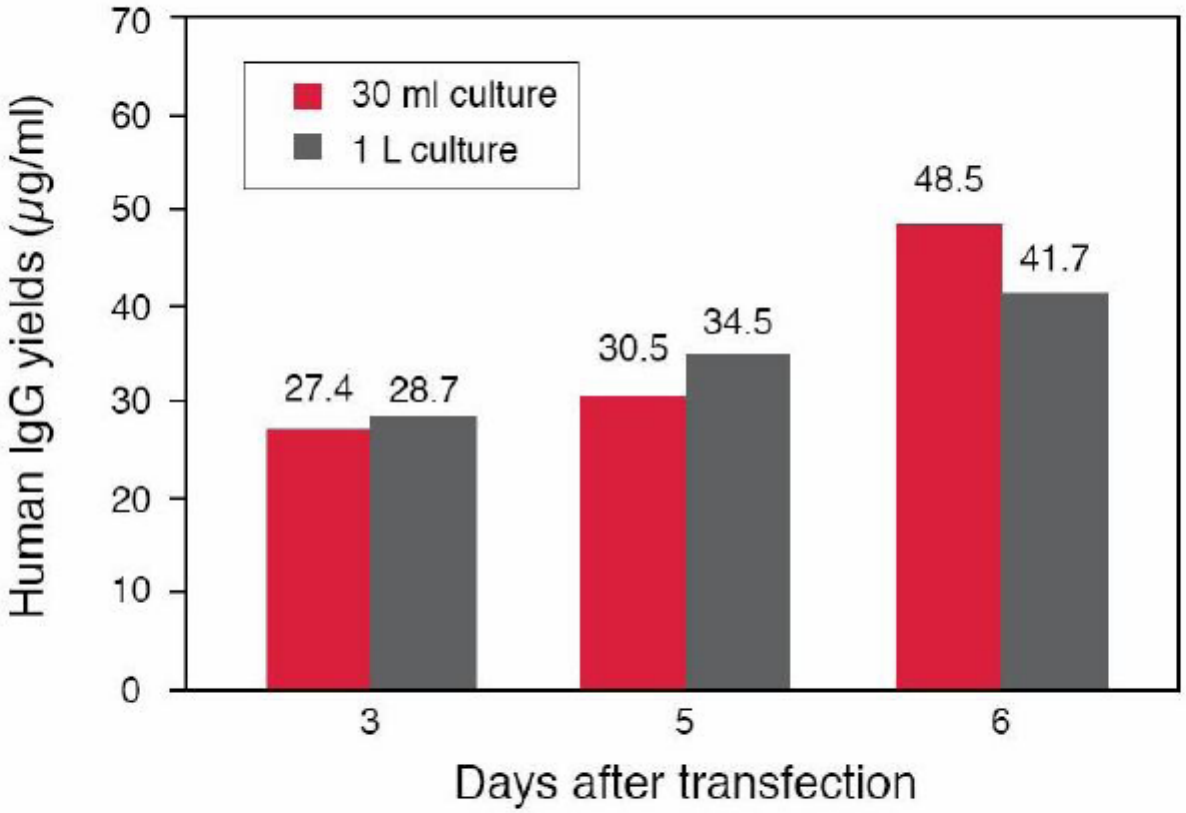
Yield versus Time & Effort

Flexibility of the FreeStyle™ Expression System



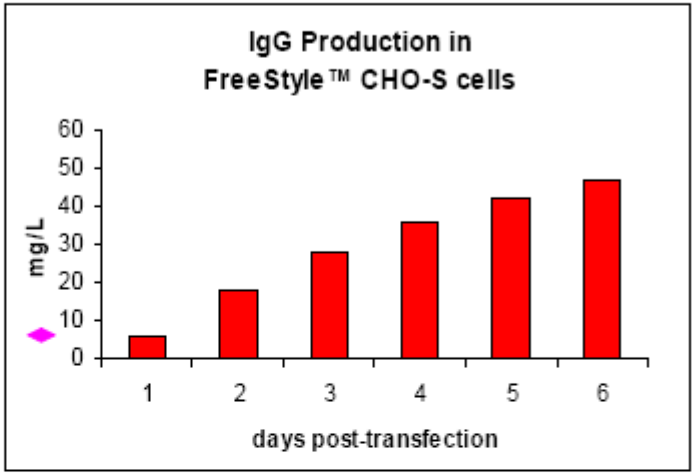
Suspension Culture = Greater Ease and Flexibility

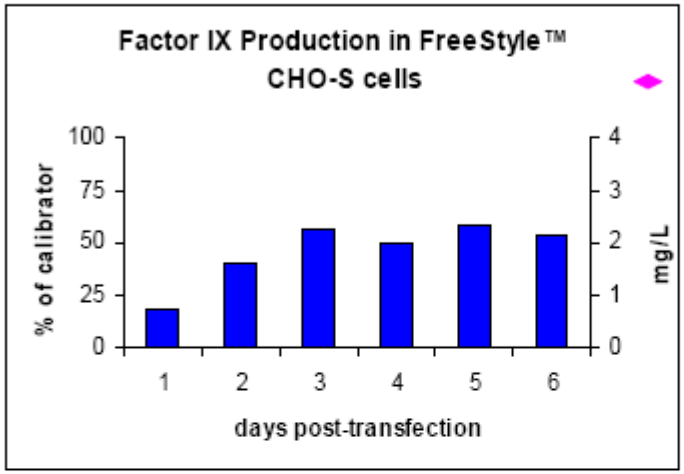
Easy Scale Up from 30mL to 10L

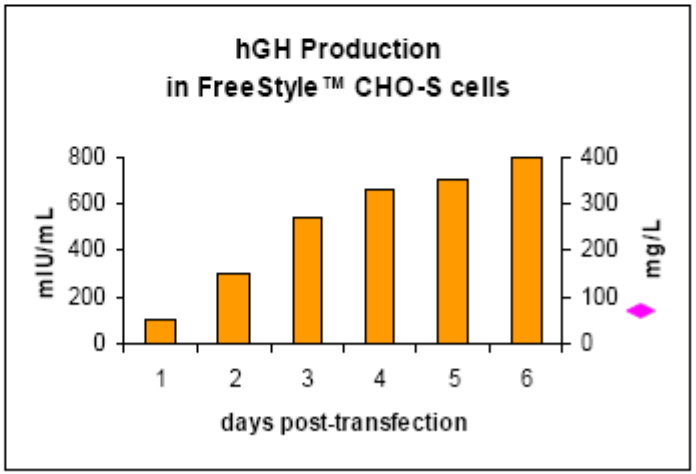


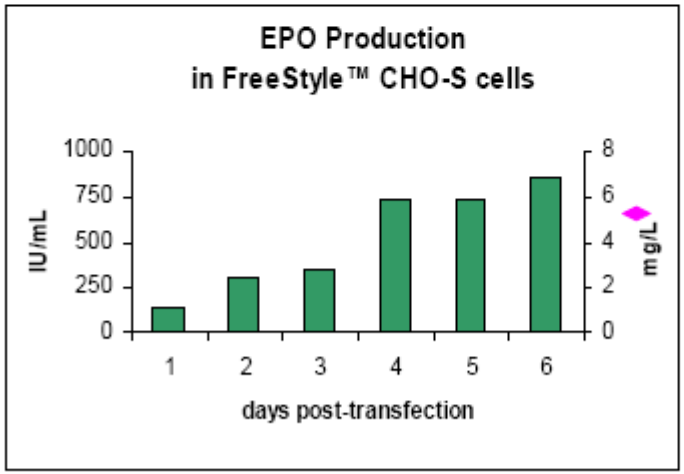
Transfection complexes were formed combining 1.25 µg/ml plasmid DNA and 1.25 µl/ml FreeStyle™ MAX in OptiPRO. Transfections were performed with a plasmid that expresses human IgG. 60% transfection efficiency at 24 hrs. Day 6 shows protein yield achieved is 45 mg/L.

FreeStyle™ MAX System - Protein Panel Study









FreeStyle™ MAX System Simple Protocol

Two Tube Protocol

1) OptiPRO™
2) DNA

3) OptiPRO™
4) FreeStyle™ MAX

10 minutes

1x10⁶ cells/mL
1-1.25 µg DNA/mL
1-1.25 µl FreeStyle™ MAX
1:1 ratio DNA:Lipid

Cell Culture Conditions

1. Maintain cell densities <1.5x10⁶/ml
2. Cell viabilities >90%
3. Regular passage schedule
4. Keep below 25-30 passages
5. Split cells 24hr prior to transfection and again on day of transfection

Cellfectin II Reagent

- Insect Cell Transfection -

Cellfectin II Reagent: Insect Cells

Important!

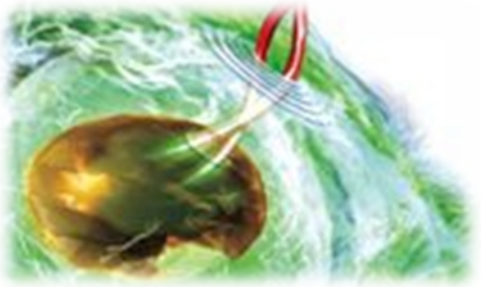
Starting on December 1, 2008, Cellfectin® reagent in this kit will be discontinued and replaced with new and improved Cellfectin® II reagent. Please refer to the manual for minor protocol changes.

Cellfectin II Reagent is a proprietary, cationic lipid formulation in membranefiltered water suitable for DNA transfection into insect cells (Sf9, Sf21, and High Five™ cells).

- Cationic lipid formulation in membranefiltered water
- Transfection of DNA into insect cells (Sf9, Sf21, and High Five™)
- Grace's Medium.
- With Cellfectin II, you do not have to remove the medium from cells and wash cells prior to adding the DNA:lipid complex to cells.
- The DNA:lipid complex formation time is shorter (~15–30 minutes) when using Cellfectin II as compared to Cellfectin reagent.

Basic Guidelines for Choosing Transfection Reagent

LIPOFECTAMINE™ 2000	Highest efficiency for many adherent cell types; both plasmid and siRNA transfection
LIPOFECTAMINE ™ LTX	Highest efficiency and lowest toxicity; for sensitive cells; for adherent and suspension cells; only plasmid transfection
LIPOFECTAMINE™ RNAiMAX	siRNA transfection; highest knockdown efficiency; lowest toxicity
FreeStyle™ MAX	For 293 and CHO suspension cells; protein production; animal origin-free
CELLFECTIN® Reagent	Insect cells
DMRIE-C Reagent	DNA, RNA, suspension cells
Oligofectamine™ Reagent	Oligonucleotides



Thank you!