

# Expi293™ PRO Expression System: A Next-Generation Expression System for Higher Yields Across a Wider Variety of Proteins

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## Abstract

The Expi293 Expression System is well known for its robust and user-friendly protocol; however, challenges remain producing proteins that express at low levels, or not at all, in the Expi293 System. Here, we introduce the next-generation Expi293 PRO Expression System along with the principles and componentry of the system that allow for higher yields (up to 4g/L), faster expression kinetics and a scalable and automation-friendly protocol.

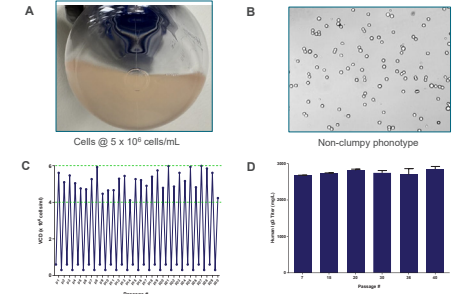
## Next Generation Expi293 - Expi293 PRO system

**Complete, optimized systems comprising:**

- New clonal cell line
- High density growth & expression media
- High efficiency transfection reagents
- Expression enhancers
- Feeds

## Expi293 PRO Cell Line

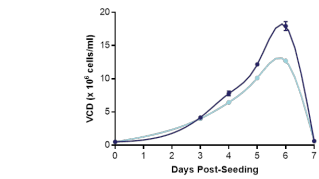
- Sub-clone derived from HEK-293 cells
- Adapted for high-density culture when paired with Expi293 PRO Medium
- Rapid growth (~24 hours doubling time)
- Non-clumping morphology
- High transfection efficiency (90-95+%)
- Robust scalability
- Stable growth and expression profiles over 40 passages



**Figure 1. Characterization of Expi293F Pro Cells**  
(A) Expi293F PRO cells in shake flask. (B) Non-clumpy phenotype. (C) Routine Cell Passaging and (D) Passage expression stability in standard shake flask culture.

## Expi293 PRO Expression Medium

- Ready to use – no supplementation needed
- Animal origin-free, serum-free, protein-free, chemically defined formulation
- Supports for high-density culture
- Same media for routine culture and transfection



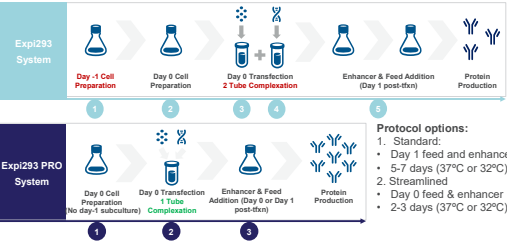
**Figure 2. Expi293 Pro Cells Growth in Expi293 Pro Expression Medium**  
Growth curves of Expi293 PRO cells grown in standard shake flask culture compared to Expi293F cells.

## Expi293 PRO Transfection Reagent Kit

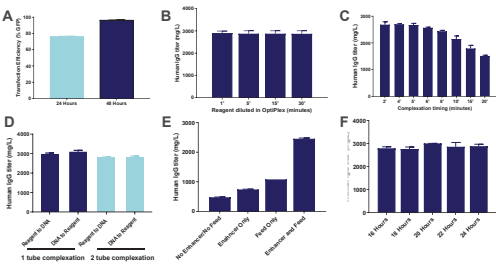
**Expi293 PRO Transfection Reagent Kit**

- One tube or two tube complexation, independent of directionality
- Transfection Reagent is stable when diluted in Opti-Plex Medium (i.e. automation protocols)
- Rapid complexation time (2-6 minutes optimal) saves hands-on time
- Next-generation enhancer & feed
- Synergistic effects allow for high-density transfections and highest titers
- Flexible timing of addition for feed and enhancer
- Feed and enhancer may be pre-mixed for ease of addition

## Expi293 PRO Protocol - Simplified and Flexible

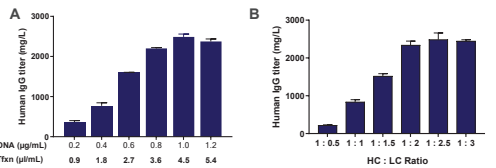


## Expi293 PRO Performance



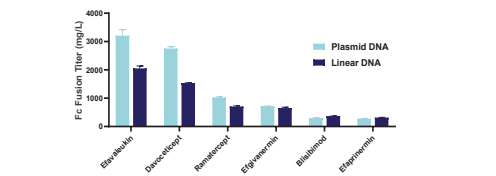
**Figure 3. Expi293 PRO transfection reagent, Feed and Enhancer**  
(A) High transfection efficiency. (B) Expi293 PRO transfection reagent is stable when diluted in Opti-Plex. (C) Rapid complexation. (D) Expi293 PRO transfection complex can be made in one tube and two tubes with both addition directions. (E) Protein titers are significantly increased with the addition of the Expi293 PRO enhancer and feed. (F) Flexibility in Expi293 PRO Enhancer and Feed additions.

## Plasmid DNA Requirement and Optimal Antibody Expression in Expi293 PRO



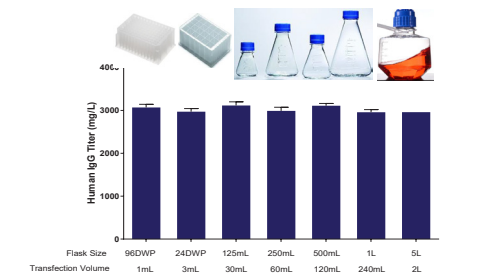
**Figure 4. Optimal plasmid DNA concentration in Expi293 PRO System**  
(A) Optimal antibody expression was observed at 1µg/mL of plasmid DNA and 4.5µL/mL of Expi293 PRO transfection reagent in Expi293 PRO system. (B) Optimal antibody expression was observed at 1:2 ratio of heavy and light chain in Expi293 PRO system.

## Linear DNA vs. Plasmid DNA in Expi293 PRO System



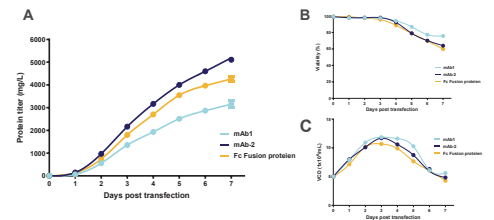
**Figure 5. Optimal plasmid DNA concentration in Expi293 PRO System**  
70% -100% of protein expression observed using linear DNA compared to traditional plasmid DNA in Expi293 PRO system

## Robust Scalability



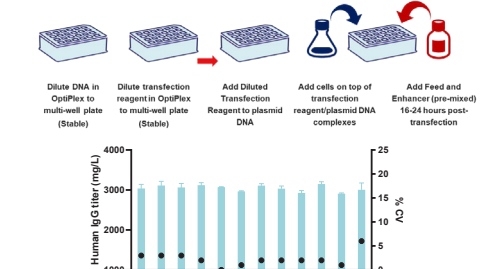
**Figure 6. Control human IgG expression across different scales**  
from 96DWP, 24DWP, 125mL, 250mL, 500mL, 1L to 5L shake flask

## Expi293 PRO Protein Expression Kinetics



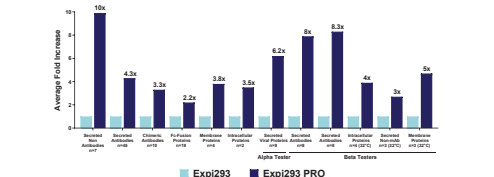
**Figure 7. Kinetics of protein expression in Expi293 PRO system**  
(A) Protein expression titer. (B) Viability post transfection. (C) Viable cell density post transfection.

## Automation Compatibility



**Figure 8. Automation - 96 Well Expression**  
Expression consistency across 12 replicate of human IgG plated in different regions of two production plates

## Higher Yields Across a Wider Variety of Proteins in Expi293 PRO

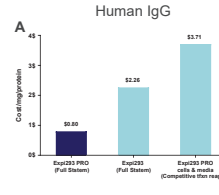


**Figure 9. Expression comparison of a wider variety of proteins in Expi293 and Expi293 PRO System**  
Secreted non-antibodies, membrane proteins, intracellular proteins, chimeric antibodies, Fc proteins, secreted antibodies and beta tester's proteins expression comparison

## What makes the Expi293 PRO system superior?

- **Unlock new protein targets**  
Express challenging and low-yield proteins
- **Highest protein yield from a transient 293 system**  
5x more protein in less volume - up to 5 g/L
- **Cost effective**  
Higher titers at the lowest cost/mg
- **Speed to protein**  
Get your protein in as little as 4 days; adjust expression duration to fit your project needs
- **High throughput-compatible**  
Simplified protocols and optimized reagents seamlessly integrate with small scale and automated platforms
- **Sustainable**  
Reduce reagents, plasticware, and labor by 3x

## Cost Effectiveness



## Sustainability

System	PEI	Expi293	Next Gen 293
Typical Titer (µg)	10 - 200 µg/mL	200 - 1000 µg/mL	800 - 4000 µg/mL
Fold Change	N/A	-4 - fold	-4 - fold
Media Required	-16L	-4L	-1L
Media Bottles	16	4	1
Shipping Cartons	3 boxes	1 box	N/A
Classification Filters	16	4	1
Purification Volume	16L	4L	1L
Vessels	1 x 50L Rucker Bag or 8 x 5L flasks	2 x 5L flasks	1 x 2L flask

**Figure 10. Cost effectiveness comparison of Expi293 and Expi293 PRO System**  
(A) Higher titer, less cost. (B) Higher Titrers = Less reagents, less cardboard, less plastic, less FTE time.