Optimized Expression of Membrane Proteins in the Expi293 and ExpiCHO Expression Systems: New Tools for Difficult to Express Proteins

Chao Yan Liu[1], Jian Liu[1], Wanhua Yan[1], Sam Stepnowski[1] ,Kyle Williston[1], Katy Irvin[1], Chetana Revankar[2], Natasha Lucki[2], Henry Chiou[2], Jonathan Zmuda[1]. Thermo Fisher Scientific, [1]Frederick, MD, U.S.A, [2]Carlsbad, CA, U.S.A;

Corresponding Author: Jon.Zmuda@Thermofisher.com

Abstract

Membrane proteins are a notoriously difficult class of proteins to express, purify and characterize. Expression levels of membrane proteins in mammalian expression systems, the preferred system to generate native-like post-translational modifications, are often so low that yields are unsuitable for the intended downstream uses. Here, we present case studies on the optimization of ion channel and GPCR expression using the Expi293 and ExpiCHO Expression Systems. Additionally, we demonstrate the utility of a new range of Expi293 reagents, including engineered Expi293 cell lines (e.g. GNTI-, Inducible and Inducible/GNTI- Expi293 cell lines) to allow for regulated expression and/or glycosylation of membrane proteins. Together, these protein expression tools significantly enhance the ability of researchers to study membrane protein biology.

Expi293 reagents to support hard-to-express proteins and structural biology applications

- Expi293 GnTI(-) Cell Line
- Expi293 Inducible Cell Line
- Expi293 Inducible GnTI(-) Cell Line
- Expi293 Methionine-Deficient Labeling

I. Expi293 GnTI(-) Cell Line

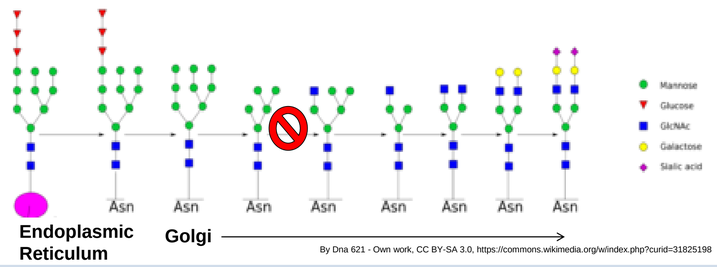

Figure 1. Typical N-linked Glycan Processing for Immunoglobulins

Man5 is the predominate glycan pattern in Expi293 GnTI (N-acetylglucosaminyltransferase I) deficient cells.

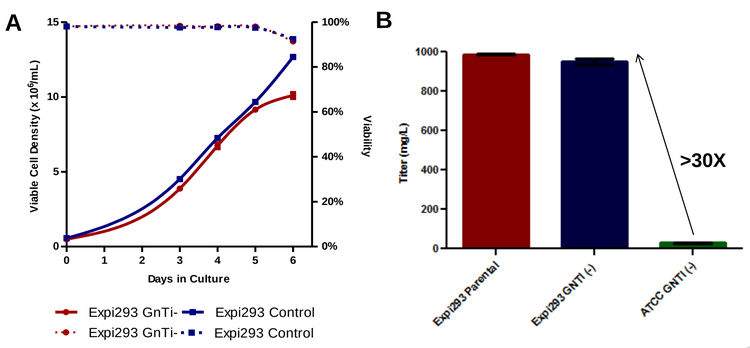

Figure 2. Growth and Expression Profiles of Expi293 GnTI(-) cells

(A) Growth kinetics of Expi293 GNTI(-) cells are comparable to parental Expi293 cells. **(B)** Expi293 GnTI(-) cells express proteins at levels comparable to parental Expi293 cells. By comparison, expression levels of ATCC GnTI(-) cells adapted into FreeStyle293 media with PEI transfection are >30-fold lower than Expi293 GnTI(-) cells in the Expi293 System.

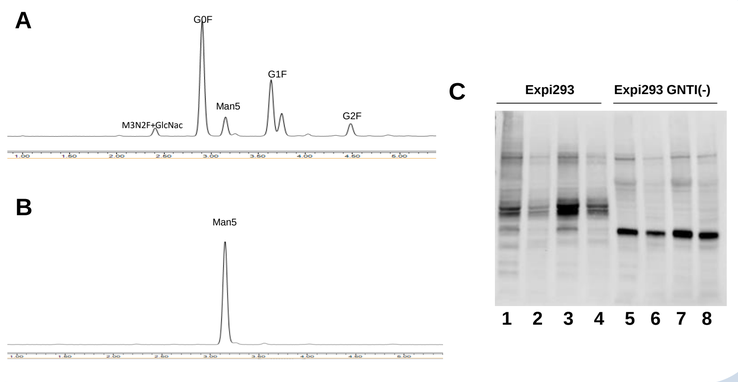

Figure 3. Glycan Patterns for Two Different Proteins Expressed in the Expi293 GnTI(-) Cell Line.

(A) Glycan patterns for human IgG expressed in Expi293 and **(B)** Expi293 GnTI(-) cell lines. Greater than 98% of glycans expressed by Expi293 GnTI(-) cells are of the Man5 variety. **(C)** Expression of the GPCR CB2 in Expi293 (Lanes 1-4) and Expi293 GnTI(-) (Lanes 5-8) cells under varying expression conditions.

II. Expi293 Inducible Cell Line

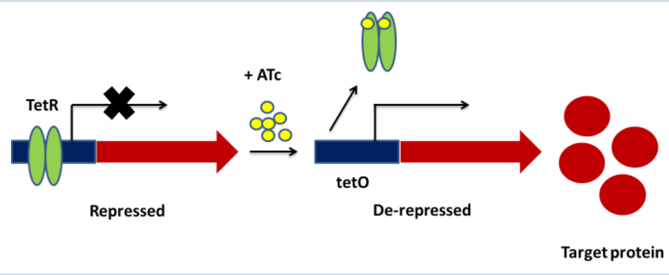

Figure 4. Principles of Inducible Protein Expression

Expi293 cells were stably transfected to express high levels of the TET repressor protein. In the absence of induction, TET repressor protein binds to the TET operator sequence of the plasmid and prevents expression of the protein of interest.

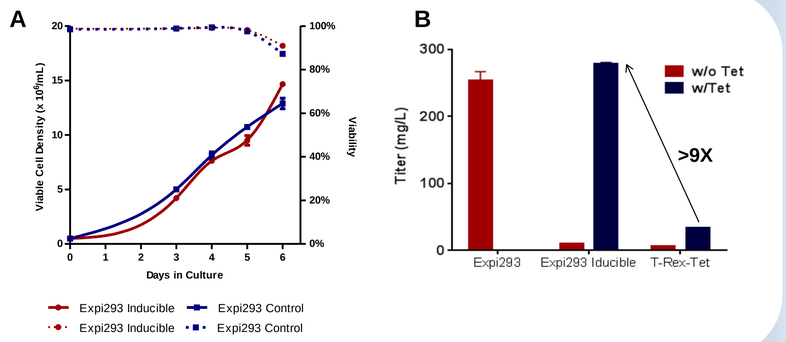

Figure 5. Growth and Expression Profiles of Expi293 Inducible cells

(A) Growth kinetics of Expi293 Inducible cells are comparable to parental Expi293 cells. **(B)** Upon full induction in pcDNA5.0/TO vector, Expi293 Inducible cells express proteins at levels comparable to parental Expi293 cells. By comparison, expression levels of T-Rex™ 293 cells adapted into FreeStyle293 media with PEI transfection are nearly 10-fold lower than the Expi293 Inducible cell line in the Expi293 System.

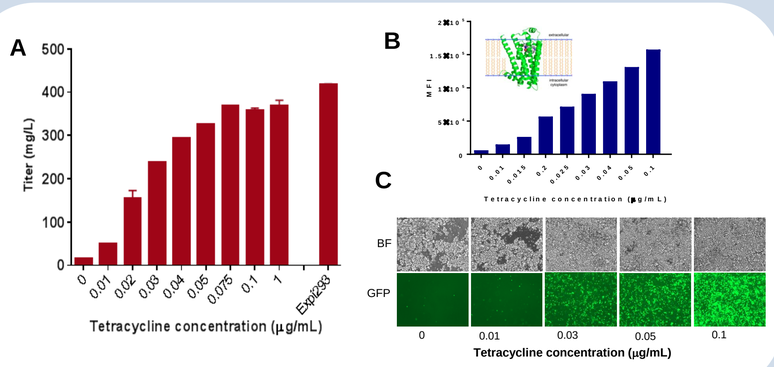

Figure 6. Modulation of Protein Expression in Expi293 Inducible Cells

(A) Cells were transfected with an Fc-fusion protein in pcDN5.0/TO vector and then induced with different levels of tetracycline in a dose-dependent manner. **(B, C)** CB2-GFP GPCR expression with different levels of induction.

III. Expi293 Inducible GnTI(-) Cell Line

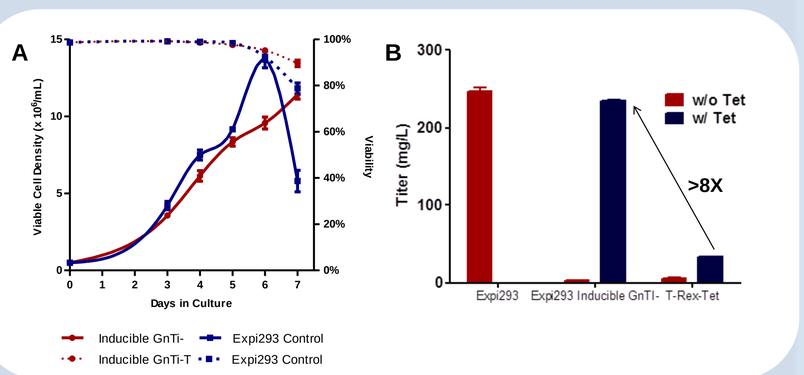

Figure 7. Growth and Expression Profiles of Expi293 Inducible GnTI(-) cells

(A) Growth kinetics of Expi293 Inducible GnTI(-) cells are comparable to parental Expi293 cells. **(B)** Upon full induction in pcDNA5.0/TO vector, Expi293 Inducible GNTI(-) cells express proteins at levels comparable to parental Expi293 cells. By comparison, expression levels of T-Rex™ 293 cells adapted into FreeStyle293 media with PEI transfection are 8-fold lower than the Expi293 Inducible GnTI(-) cell line in the Expi293 System.

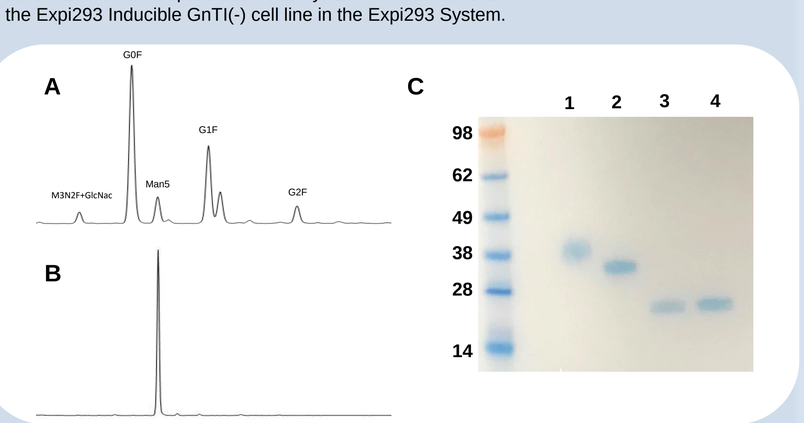

Figure 8. Glycan Patterns for Human IgG and Erythropoietin Expressed in the Expi293 Inducible GnTI(-) Cell Line.

(A) Glycan patterns for human IgG and erythropoietin (epo) expressed in Expi293 and **(B)** Expi293 Inducible GnTI(-) cell lines. **(C)** SDS PAGE of: 1) native epo, 2) epo expressed in the Expi293 Inducible GnTI(-) cell line, 3) PNGaseF deglycosylated epo from Expi293, 4) deglycosylated epo from Expi293 Inducible GnTI(-) cells.

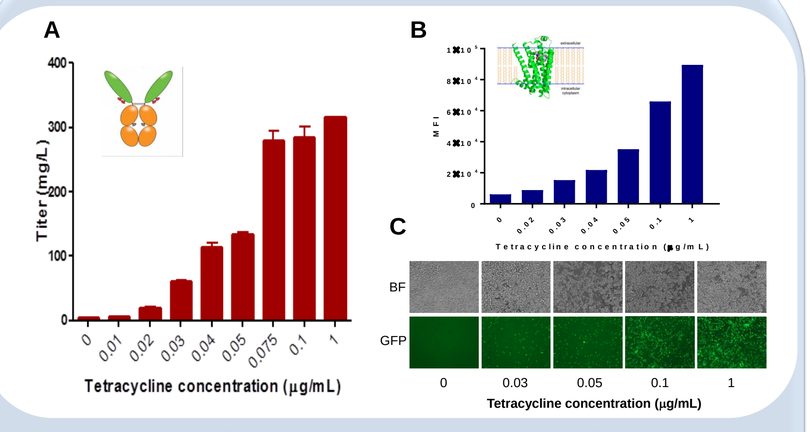

Figure 9. Modulation of Protein Expression in Expi293 Inducible GnTI(-) Cells

(A). Cells were transfected with a plasmid encoding an Fc-fusion protein in pcDN5.0/TO vector and then induced with different levels of tetracycline. (B,C) CB2-GFP GPCR expression with different levels of induction.

IV. Optimization of AA2AR GPCR Expression

Cell Line	DNA	Enhancer (1)	Feed (Enhancer 2)	Significant Factor
Expi293	0.25ug/mL **0.5ug/mL** 1.0ug/mL	0% 50% 100% 150%	0% 50% 100% 150%	**DNA (PValue >0.000001)** Enhancer (PValue 0.00001)
GnTI(-)	0.25ug/mL 0.5ug/mL **1.0ug/mL**	0% 50% 100% 150%	0% 50% 100% 150%	**DNA (PValue >0.000001)** Enhancer (PValue 0.00028)
ExpiCHO	0.25ug/mL 0.5ug/mL 1.0ug/mL	**0%** 50% 100% 150%	0% 50% 100% 150%	**Feed (PValue >0.000001)** Enhancer (PValue >0.000001)

Figure 10. Optimization of GPCR expression in the Expi293, Expi293 GnTI(-), and ExpiCHO expression systems

(A) AA2AR GPCR expression was optimized by titrating plasmid DNA, Feed and Enhancers in Expi293, Expi293 GnTI(-), and ExpiCHO cells. AA2AR was harvested on days 2 and 3 post-transfection and analyzed by FACS. The mean fluorescence intensity (MFI) was multiplied by the viable cell density (VCD) at the time of harvest. The data revealed significant factors for each expression system.

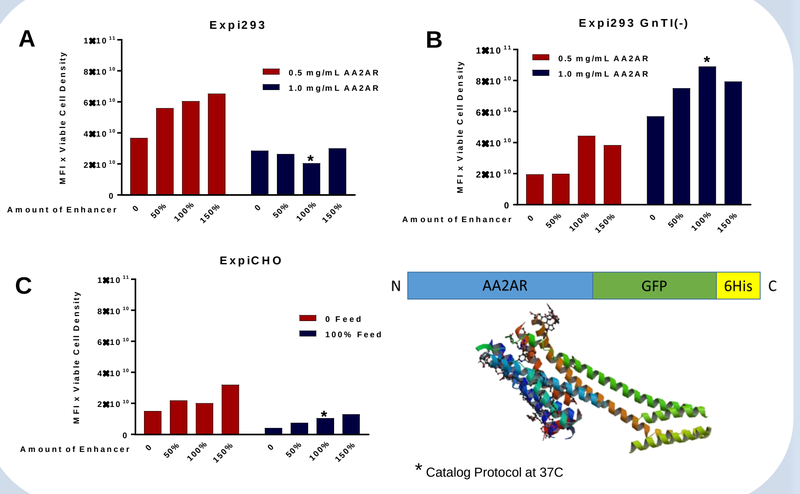

Figure 11. AA2AR GPCR expression levels in the Expi293, Expi293 GnTI(-), and ExpiCHO expression systems

(A) AA2AR GPCR optimized expression in the Expi293 Expression System; significant factors for optimized expression were DNA (0.5mg/mL) and enhancer. **(B)** AA2AR GPCR optimized expression in the Expi293 GnTI(-) Expression System; significant factors for optimized expression were DNA (1.0mg/mL) and enhancer. **(C)** AA2AR GPCR optimized expression in the ExpiCHO Expression System; significant factors for optimized expression were feed and enhancer.

VI. Expression of AA2AR in Expi293 Inducible Cells

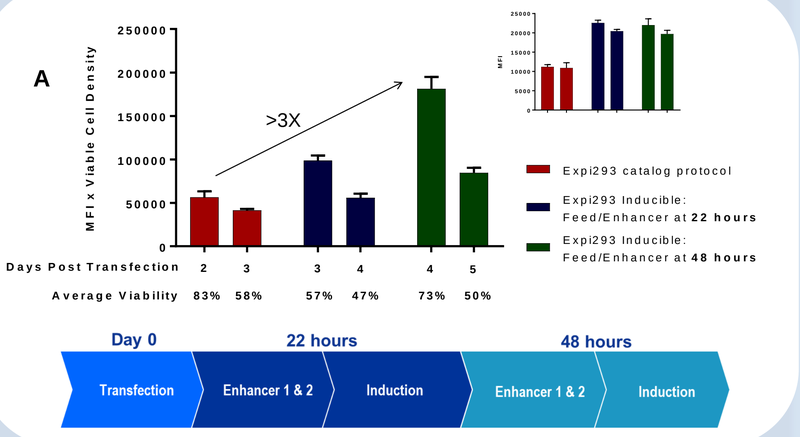

Figure 12. AA2AR expression in Expi293 Inducible cells

(A) AA2AR expression in Expi293 cells or Expi293 Inducible cells. Expi293 inducible cells were induced at two time points along with feed and enhancer. AA2AR was harvested on day 2 and 3 post-transfection for Expi293 cells and day 2 and 3 post-induction for Expi293 Inducible cells. The mean fluorescence intensity (MFI) was multiplied by the viable cell density (VCD) at the time of harvest. Inset: MFI, not considering viable cell density.