Automating Expi293™ PRO Expression System for mAb and Fc Fusion Protein Production

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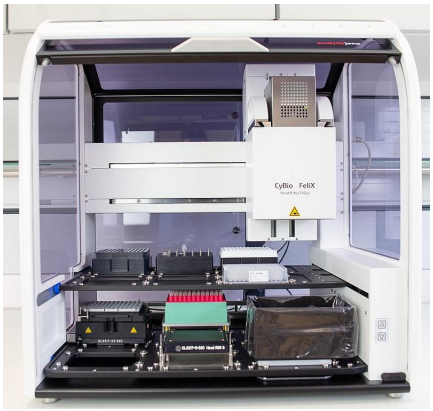
Abstract

Automation of small scale, high-throughput protein expression is becoming more critical as artificial intelligence (A.I.) and machine learning (M.L) are increasingly being implemented into the design and optimization of therapeutic protein candidates. Here, we describe key steps involved in automating the upcoming Expi293 PRO™ Expression System to produce various classes of therapeutic proteins, including recommendations for a streamlined protocol as well as important guidance that should be adhered to for optimal performance.

Transfection Materials
Gibco™ Expi293™ PRO Expression System Kit
Expi293™ PRO Cells
Fisherbrand™ 96-Well DeepWell™ Plate Polypropylene 1 mL (Cat. #: 12566611)
Fisherbrand 96-Well DeepWell Plate Polypropylene 2 mL (Cat. #: 12566612)
Thomas Scientific AeraSeal Film, Sterile (Cat. #: NC0884352)
CO_2 resistant shaker with 3 mm orbit (Cat.#: 88881103)

Liquid Handling Materials
CyBio FeliX Basic Unit Liquid Handling Platform
CyBio FeliX Head R 96/1000 µL
CyBio RoboTipTray 96/1000 µL
CyBio Head R96 8 Channel adapter OL3317-11-330
Axygen 12 Channel Reservoir
Axygen Single Well 96 Bottom High Profile Reservoir
1000 µL Clear Hanging Tips
CyBio 1000 µL Hanging Tip Tray OL3317-11-105



Enhancing DNA Delivery with Expi293 PRO

Testing Transfection methods for Automation Flexibility

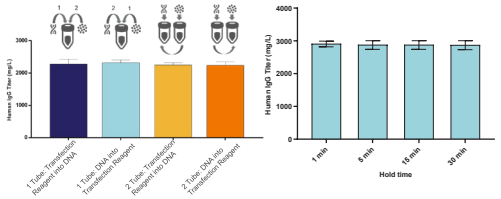


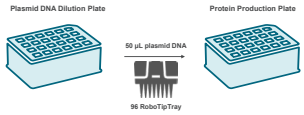
Figure 1. A. Stability of transfection methodologies combining the reagent with DNA using one-tube (dark and light blue bars) versus two-tube (yellow and orange bars) methods. The effect of adding DNA first versus the transfection reagent was also evaluated in each of the methods. **B.** Stability of the transfection reagent in OptiPlex at room temperature over 30 minutes. Aliquots were taken at 1, 5, 15, and 30 minutes to transfect Expi293 cells, and human IgG titers were measured on Day 7.

Table 1. Volumes Used for Transfection

	Per Well	Per 96 Well Plate Automation (incl. overage)
Number of Cells Required	5.0 × 10^6	6.45 × 10^8
Volume to Transfect	1 mL	120 mL
Amount of Plasmid DNA	1.0 µg total plasmid DNA per mL of culture volume to transfect	
Volume of Plasmid DNA	1.0 µL	N/A
Volume of Opti-PLEX™ for DNA dilution	50 µL	N/A
Expi293™ PRO Transfection Reagent	4.5 µL	900 µL
Volume of Opti-PLEX™ for Transfection Reagent Dilution	50 µL	10 mL
Expi293™ PRO Enhancer	6 µL	6 mL
Expi293™ PROFeed	80 µL	80 mL

I. Plasmid DNA Preparation and Plating

1. Pre-mix heavy and light chain plasmid DNA at a 1:2 HC:LC ratio in a 1 mL 96 well **Plasmid DNA Stock Plate**
2. Dilute Plasmid DNA 1:50 in Opti-PLEX™
3. Add 50 µL diluted DNA from Step 2 to each well of a 2 mL 96 DeepWell **Protein Production Plate**

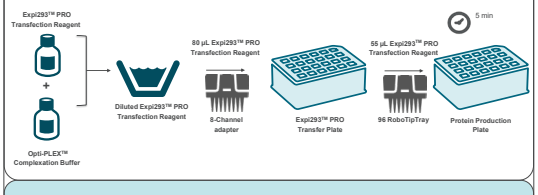


Tips and Watch-outs:

1. Ensure plasmid DNA heavy:light chain ratio is optimal for your antibody constructs
2. One 96 RoboTipTray may be used for all Opti-PLEX™ additions assuming tips do not come into contact with the contents of the wells

II. Transfection Reagent Preparation/Plasmid DNA Complexation

1. For one 96 DeepWell, dilute 909 µL of Expi293™ PRO Reagent with 9.09 mL of Opti-PLEX™ Complexation Buffer
2. Add 80 µL of diluted Expi293™ PRO Reagent to each well of a 1 mL 96 well **Transfer Plate**
3. Transfer 55 µL of diluted Expi293™ PRO Reagent to each well of the **Protein Production Plate** (containing the diluted plasmid DNA) using a 96 RoboTipTray. Mix once by aspiration and allow to complex

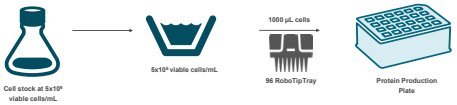


Tips and Watch-outs:

1. The Expi293™ PRO Reagent diluted in Opti-PLEX™ is stable for up to 30 minutes
2. Ensure significant reagent overage for simultaneous addition to the Protein Production Plate
3. Target plasmid DNA complexation time at 5 minutes prior to addition of cells

III. Addition of Cells

1. For each 96 DeepWell Plate, prepare 120 mL of cell stock at a final density of 5x 10^6 viable cells/mL
2. Add 1 mL of cells to each well of the **Protein Production Plate** containing transfection reagent/plasmid DNA complexes
3. Cover the **Protein Production Plate** with a gas permeable sealer and incubate at 37ºC in a humidified incubator with 8% CO_2 on a 3 mm orbital shaker at 1000 RPM



Tips and Watch-outs:

1. Cells should be mixed to ensure a homogenous suspension prior to addition to the Protein Production Plate
2. Large volume addition of cells to the transfection reagent/plasmid DNA complexes ensures sufficient mixing without additional aspiration and dispense steps

IV. Addition of Feed and Enhancer

1. Combine 80 mL Expi293™ PRO Feed and 6 mL Expi293™ PRO Enhancer.
2. 18 hours post-transfection, add 86 µL of Expi293™ PRO Feed/Enhancer mixture to each well of the **Protein Production Plate**
3. Re-cover the **Protein Production Plate** with a gas permeable seal and incubate at 37ºC (or 32ºC depending on protein) in a humidified incubator 8% CO_2 on a 3 mm orbital shaker at 1000 RPM

Tips and Watch outs:

1. Feed and Enhancer can be premixed before adding to reservoir
2. One 96 RoboTipTray may be used for all Feed and Enhancer additions assuming tips do not come into contact with the contents of the wells
3. Use a humidified incubator to minimize evaporation in the wells during production

Results

Antibody 1 Control Blocks

Averages of Panel Titers

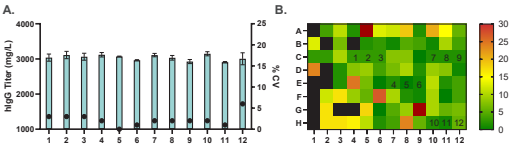


Figure 2. A. Human IgG protein expression consistency across 12 replicate expressions of Antibody 1 plated in different regions of two Protein Production Plates. **B.** A Coefficient of Variation (% CV) of <30% for all expressed proteins across 2 Protein Production Plates printed from the same DNA Dilution Plate. Antibody 1 controls wells are identified in wells 1-12. *Note: Black blocks indicate foamed out wells.*

Antibody Cross-Plate Consistency

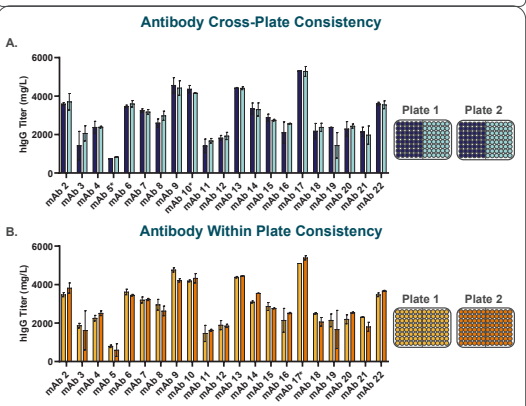


Figure 3. This figure presents a comparison of human IgG titers evaluating sample distribution consistency between and within plates. Each plate was divided into two regions, with samples collected from both halves. **A.** Comparison of monoclonal antibodies from the first half of Plate 1 and Plate 2 (dark blue) and from the second half of Plate 1 and Plate 2 (light blue). **B.** Comparison of monoclonal antibodies within Plate 1 between the first and second half (yellow), and within Plate 2 between the first and second half (orange). *Note: Any protein indicated with * had one value omitted due to one of the wells in the comparison foaming out as indicated in Figure 2D.*

Fc Fusion Cross-Plate Consistency

Fc Fusion Within Plate Consistency

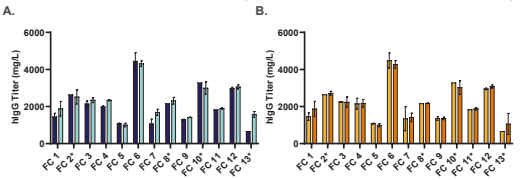
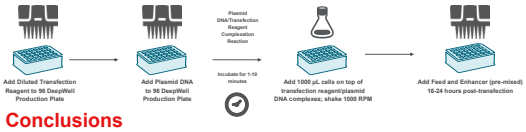


Figure 4. This figure presents a comparison of human IgG titers evaluating sample distribution consistency between and within plates. Each plate was divided into two regions, with samples collected from both halves. **A.** Comparison of Fc fusion proteins from the first half of Plate 1 and Plate 2 (dark blue) and from the second half of Plate 1 and Plate 2 (light blue). **B.** Comparison of Fc fusion proteins within Plate 1 between the first and second half (yellow), and within Plate 2 between the first and second half (orange). *Note: Any protein indicated with * had one value omitted due to one of the wells in the comparison foaming out as indicated in Figure 2B.*

High Throughput Expi293 PRO Automation Protocol

1. Pre-mix heavy and light chain plasmid DNA at a 1:2 HC:LC ratio in a 1 mL 96 well plate
2. Dilute plasmid DNA 1:50 in Opti-PLEX
3. Dilute Expi293 PRO Reagent 1:10 with Opti-PLEX Complexation Buffer and add 55 µL to each well of a 96 DeepWell Protein Production Plate
4. Transfer 50 µL of diluted plasmid DNA from step 2 to each well using a 96 RoboTipTray, mix once by aspiration, and allow to complex for 5 min
5. Prepare 120 mL of cell stock at 5x10^6 viable cells/mL and add 1000 µL to each well of the Protein Production Plate
6. Cover the plate with a gas permeable sealer and incubate at 37ºC, 8% CO_2, 1000 RPM
7. After 18 hours post-transfection, combine 80 mL Expi293™ PRO Feed with 6 mL Expi293™ PRO Enhancer, then add 86 µL to each well; re-cover and incubate



Conclusions

Here, we demonstrate the principles for automating the Expi293™ PRO Expression Systems for the purposes of expressing monoclonal antibodies and Fc fusion proteins. Utilizing these principles, excellent reproducibility may be obtained across production runs. Lowering the Cell transfection volume from 1 mL to 800 µL and lowering the shake speed to 900 RPM may be beneficial in avoiding foaming.