

# Advancing the development of therapeutics against infectious diseases with optimized protein expression systems

## Introduction

Novel and emerging infectious diseases can be a serious threat to human health and the global economy. To mitigate these threats, it is necessary to initiate comprehensive containment strategies such as virus characterization, serological testing, therapeutic antibody research, and vaccine development. The global crisis caused by the SARS-CoV-2 virus amplified the need to accelerate diagnostics, therapeutics, and vaccine development, spurring new and efficient workflows to combat rapidly evolving pathogens. In recent years, these workflows have harnessed the benefits of protein expression systems for efficient infectious disease research.

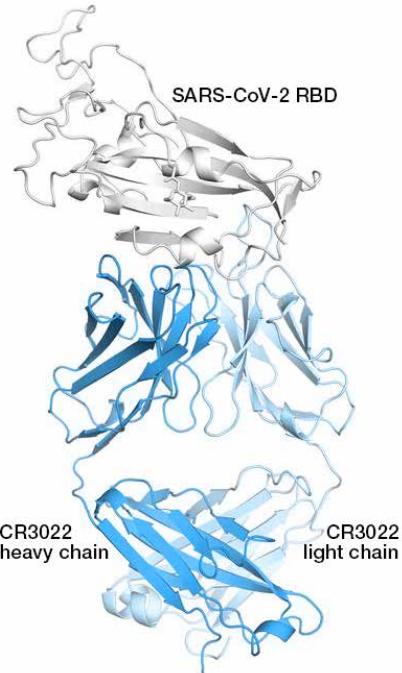
Transient protein expression can be employed to further infectious disease research, specifically to produce antigens to study viral structure and function. Protein expression systems also assist in the development of serological assays that are vital to understanding antibody response and viral pathogenesis. In addition, therapeutic antibodies that neutralize viruses and limit spread can be produced using transient expression workflows. This enables rapid production of protein panels to test for the most efficacious antibody. Recombinant vaccines, which utilize antigens to induce immunity against pathogens, also use protein expression systems for antigen production.

Evolving strategies like these for therapeutics development have driven innovation in the protein expression space, giving rise to chemically defined systems for rapid, high-yield protein production for many applications. Gibco™ transient protein expression systems are optimized to provide superior protein yields and reproducibility, typically obtained with stable cell lines, while maintaining the simplicity, speed, and ease of use associated with traditional transient expression workflows. The suite of Gibco protein expression systems, available in both mammalian (HEK293, CHO-S) and insect (Sf9) cell line formats, offers integrated solutions to fuel infectious disease research and mitigate extensive viral spread.

## Virus structure

Many structural biologists utilize protein expression platforms to generate sufficient material for crystallization and cryo-electron microscopy (cryo-EM) experiments. The global crisis caused by SARS-CoV-2 highlights the importance of expediting viral structure studies with optimized protein expression systems.

For instance, the Gibco™ Expi293™ Expression System Kit enables high-yield expression of recombinant viral capsid proteins. Yuan et al. determined the crystal structure of the receptor-binding domain (RBD) of the SARS-CoV-2 spike (S) protein in complex with CR3022, a neutralizing antibody isolated from a convalescent SARS-CoV-infected patient (Figure 1) [1]. CR3022 targets an epitope highly conserved between SARS-CoV-2 and the previously identified coronavirus SARS-CoV, but it binds more tightly to SARS-CoV because it contains a glycan that is absent in SARS-CoV-2. Structural analyses such as these, fueled by Gibco expression system kits, provide molecular insights into SARS-CoV-2 antigenicity, antibody recognition, and therapeutics research, highlighting the need for optimized protein expression systems to support a broader range of structural applications. The Expi293 system is now equipped with additional capabilities, including glycan modulation, inducible expression, and methionine labeling, to further accommodate researchers' critical structural requirements.



**Figure 1. Structure of CR3022 complexed with SARS-CoV-2 RBD.** The heavy chain (blue) and light chain (light blue) of CR3022 are shown bound to the RBD of the SARS-CoV-2 spike protein (silver). Illustration adapted from Yuan et al. [1], under terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license, creativecommons.org/licenses/by/4.0/.

Gibco protein expression systems also enable biologists to transition their infectious disease research to bioproduction. For example, Hsieh et al. characterized 100 structure-guided designs of the SARS-CoV-2 S protein to identify 26 unique substitutions that increased protein yield and stability [2]. They identified HexaPro, an S protein variant with the ability to withstand heat stress and retain prefusion spike conformation. To assess the viability of HexaPro as a potential antigen or diagnostic reagent, they analyzed large-scale production with the Gibco™ ExpiCHO™ Expression System Kit and the Gibco™ FreeStyle™ MAX 293 Expression System. They concluded that large-scale production of a stabilized prefusion S protein could accelerate vaccine development and serological diagnostics for SARS-CoV-2.

### Serological assays

Serological assays identify antibodies and antigens in patient serum, indicating if a patient has previously contracted a disease. The crisis caused by the SARS-CoV-2 virus has stressed the need for effective serological testing to gain insight into the full extent of viral spread and pathogenicity.

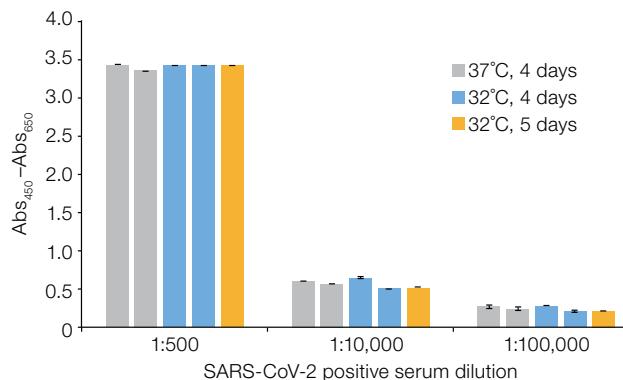
Stadlbauer et al. established a detailed protocol for the expression of SARS-CoV-2 antigens in a two-stage enzyme-linked immunosorbent assay (ELISA) [3].

This assay can be used in both research laboratories and clinical research settings to understand the antibody responses mounted against SARS-CoV-2. In the protocol, full-length S proteins and RBDs are expressed in the Expi293 system, purified via gravity flow, and used to coat ELISA plates. Human serum samples are then measured against the RBD, which is expressed well in Gibco™ Expi293F™ Cells. Positive samples then undergo a confirmatory ELISA against the S protein, which is more challenging to express in Expi293F Cells than RBD. Because serological tests require high titers of the assay-specific S protein, the S protein is a limiting factor for SARS-CoV-2 serological assays.

To meet the demand for this antigen, Esposito et al. investigated several recombinant protein expression variables to improve the yield of the S protein in transient expression systems [4]. Their optimization of the protein purification process from the transient Expi293 system produced sufficient S protein (up to 6.4 mg/L) from a single 4 L expression run to support a serosurvey led by the NIH (Table 1). Their work helped elucidate the extent of the coronavirus immune response and demonstrated that transient expression is a robust method for reliable protein production (Figure 2).

**Table 1. Effect of expression and purification parameters on spike protein yield.** The post-harvest production process consisted of tangential flow filtration, immobilized metal affinity chromatography (IMAC), and a desalting column. Yields are from independent experiments (nd: not determined) [4].

Condition	Vaccine Research Center (mg/L)	Mt. Sinai (mg/L)
37°C/72 hr IMAC/SEC	0.3	0.9
37°C/96 hr IMAC/desalt	2.0, 2.0, 1.4	1.7, 2.6
37°C/96 hr IMAC/desalt	4.8, 5.2, 6.1	4.6, 5.3, 5.2
37°C/120 hr IMAC/desalt	6.4, 5.0	4.4
37°C/96 hr MagBeads/desalt	4.1, 5.5	nd



**Figure 2. ELISA sensitivity of independent batches of spike protein produced at various concentrations, expression times, and temperatures.** Consistent lot-to-lot performance of the spike protein was achieved—an essential component of a high-quality, sensitive serological assay. Adapted from Esposito et al. [4].

## Therapeutic antibodies

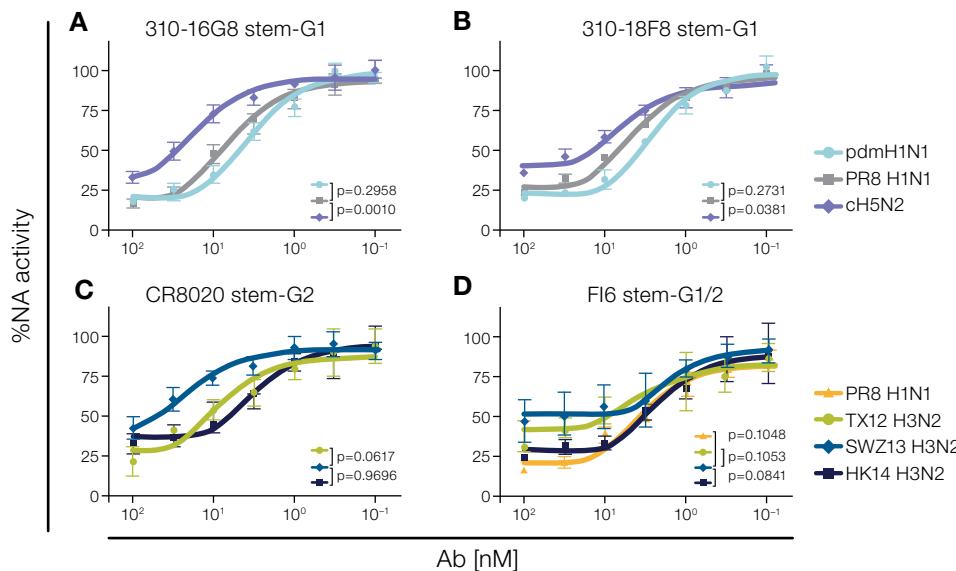
Identifying antibodies that can act as antivirals is an important strategy in the fight against emerging viruses. For instance, Pan et al. have shown that a recombinant SARS-CoV-2 S protein RBD can bind to the SARS-CoV-2 receptor, triggering an *in vivo* antibody response via RBD-specific F(ab')<sub>2</sub> with a potent inhibitory effect on SARS-CoV-2 [5]. Their high-yield production of the recombinant RBD was accelerated using the ExpiCHO expression system, which allowed the researchers to prioritize receptor blockade assays, virus neutralization tests, and biomolecular interaction analyses to rapidly identify a key antibody against SARS-CoV-2.

In addition to supporting research on therapeutics against emerging viruses, protein expression systems assist researchers in identifying novel treatment mechanisms for prevalent infectious diseases such as influenza. Historically, researchers have targeted the influenza virus hemagglutinin (HA) protein with broadly neutralizing antibodies (bNAbs). However, using the Expi293 expression system, Kosik et al. demonstrated that anti-HA stem antibodies (Abs) can also inhibit virus release by blocking neuraminidase (NA) activity against large substrates (Figure 3) [6]. The use of the Expi293 expression system helped to rapidly identify the potential therapeutic synergy between NA inhibitors and anti-stem mAb treatment.

## Vaccine candidates

Rapid antigen production is critical for efficiently screening and characterizing lead vaccine candidates. Transient protein expression offers a flexible and economical alternative to the time-consuming and costly process of generating and selecting stable cell lines, enabling scientists to identify and produce potential vaccine candidates more effectively and efficiently.

For years, designing a trimer-based HIV-1 vaccine has been a challenge due to the metastable nature of the envelope glycoprotein. He et al. evaluated the metastability of the envelope glycoprotein by comparing the expression of native-like trimers in Gibco™ ExpiCHO-S™ cells and Expi293F cells [7]. Expression of the glycoprotein gp140 trimer in ExpiCHO-S cells led to superior yield, purity, and antigenicity compared to its expression in Expi293F cells. The researchers estimated that the gp140 yield from 100 mL of the ExpiCHO-S system was equivalent to that of the yield from 2–4 L of the Expi293F system. In addition, there was a substantial reduction of misfolded species in the envelope protein obtained from the ExpiCHO-S cells, compared to the Expi293F cells. These results demonstrate that expression systems are protein- and application-dependent. In the case of He et al., the advantages of the ExpiCHO system resulted in robust production of native-like gp140 trimers, which enabled elucidating virus evasion tactics and antibody responses for further vaccine development research.

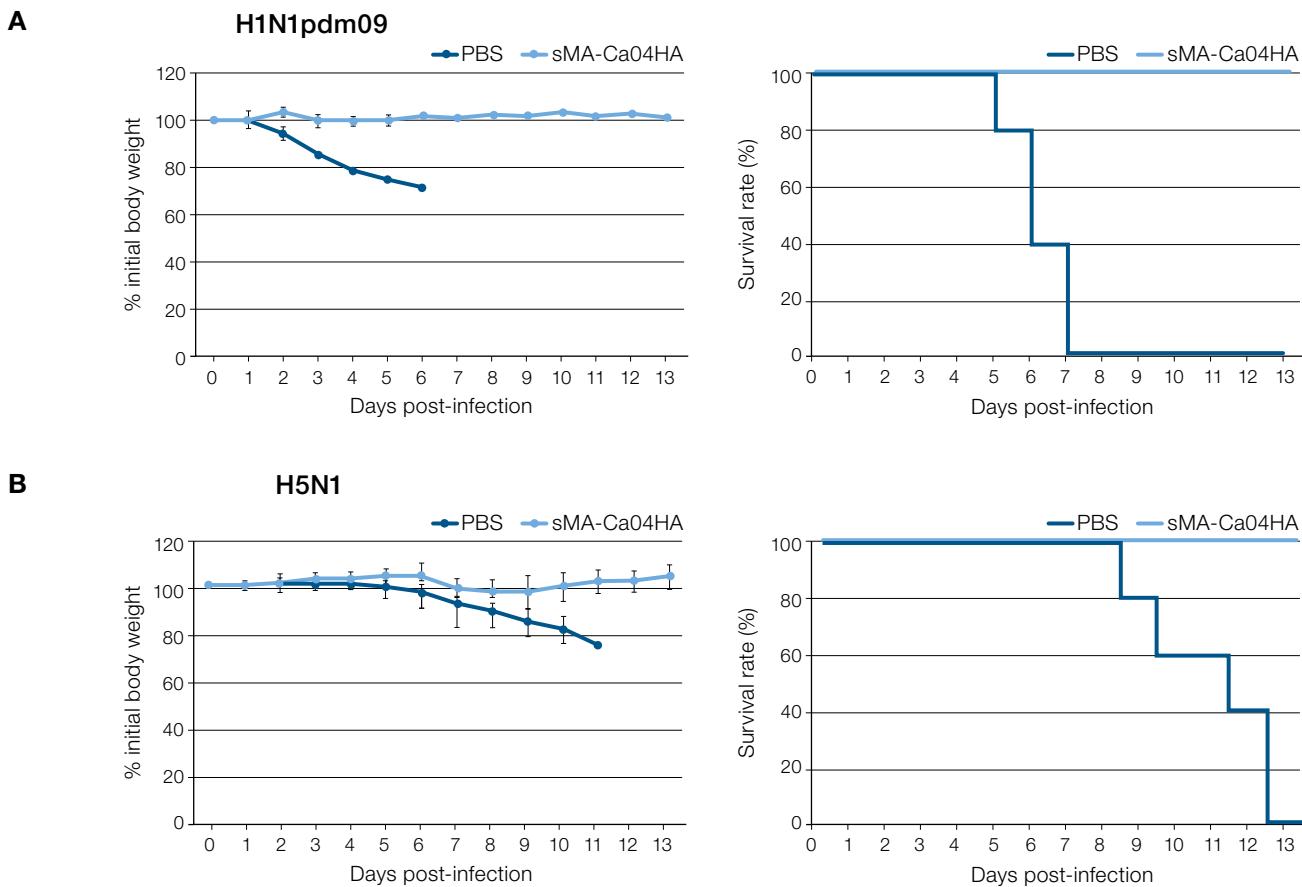


**Figure 3. Anti-stem mAbs efficiently inhibit NA activity.** An enzyme-linked lectin assay (ELLA) was performed to measure the capacity of purified anti-stem mAbs on NA activity against several viruses. The %NA activity was normalized to the absence of the Ab (set to 100%). Anti-stem mAbs 310-16G8 (**A**) and 310-18F8 (**B**) are specific to group 1 (G1) HA stems and efficiently block N1 or N2 NA activity using viruses with H1 HA stems: PR/8/34 (H1N1), Cal/4/09 (H1N1), and chimeric cH5N1 (H5-head-H1-stem N2). Anti-stem mAb CR8020 (**C**) is specific to group 2 (G2), and Fl6 (**D**) is specific to both groups (G1/2); the mAbs inhibit NA activity on multiple group 2 viruses, indicating that NA can be inhibited by a G1/2 cross-reactive stem-specific Ab ( $n = 6$ ) [6]. Figure used with permission from Rockefeller University Press.

In addition to accelerating the vaccine candidate process, the right protein expression system can significantly increase the immunogenicity of recombinant proteins. For instance, the influenza HA protein, a successful vaccine target against the influenza virus, has been produced recombinantly in several different systems over the years. When Yamada et al. expressed recombinant HA protein in Expi293F Cells, they found that it protected mice against homosubtypic H1N1pdm09 and heterosubtypic H5N1 by inducing high levels of neutralizing antibodies against recombinant HA protein sMA-Ca04HA (Figure 4) [8]. This work indicates that intramuscular immunization with recombinant HA proteins may offer a new strategy for influenza vaccine development by utilizing Expi293F cell-based vaccines.

## Conclusions

Transient protein expression systems enable high-yield, consistent protein production for a diverse array of downstream applications. The need for rapid protein production is especially evident in infectious disease research, where workflows must be optimized to generate high-quality proteins for structural research, serological testing, therapeutics discovery, and vaccine candidate screening. Emerging infectious diseases drive innovation in the protein expression space, accelerating the development of new platforms that offer integrated solutions to understand viral pathogenicity and spread.



**Figure 4. Change in body weight (left) and survival rate (right) of mice after virus challenge post-immunization.** Mice were first mock-immunized with PBS (control) or immunized with sMA-Ca04HA. Two weeks post-vaccination, the mice were challenged with 10 MLD<sub>50</sub> of (A) H1N1pdm09 and (B) H5N1 (n = 5). MLD = minimum lethal dose. Figure adapted from Yamada et al. [8], under terms of the Creative Commons Attribution license (CC BY 4.0), creativecommons.org/licenses/by/4.0/. © 2019 Yamada, Yasuhara and Kawaoka.

## References

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## Other resources

- Transient Protein Expression Platforms
- ExpiCHO Expression System
- Expi293 Expression System
- ExpiSf Baculovirus Expression System
- Protein Expression Publication Hub
- Gibco Protein Expression Basics

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