WHITE PAPER

Achieving higher throughput for the bioanalysis of advanced biotherapeutics

Advanced biotherapeutics require characterization and stringent quality control. Predictions on drug efficacy, potency, and stability can be improved by assessing critical quality attributes early on during the cell line development process, and select the best molecules to take forward into the clinic.

The CHOSOURCE expression platform

At Revvity, Neha Mishra, Product Specialist in BioProduction, utilizes clone expression systems to generate monoclonal and bispecific antibodies. Having had hands-on experience with cell line development, she also understands the advanced need of researchers.

Revvity's CHOSOURCE™ expression platform has been used to develop and manufacture biotherapeutics, and can be combined with existing processes across commercial manufacturing workflows. The CHOSOURCE expression platform includes the CHOSOURCE GS KO cell line which is a CHO-K1 suspension adapted cell line with the glutamine synthetase gene (GS) knocked out (KO), providing a robust industry standard selection system.

Recently, the CHOSOURCE platform has been enhanced with the introduction of the CHOSOURCE TnT transposon technology. The TnT transposon system includes the TnT transposon vector and the transposase mRNA. The transposon vector contains a dual expression cassette allowing for the expression of up to two genes of interest (GOI). Both vector and mRNA are co-transfected into a CHOSOURCE host cell line, such as the GS KO cell line. Within the cell, the transposase mRNA is translated to enzyme which then binds to terminal inverted repeats in the vector and eventually excises the transgene from the vector for incorporation into the genome, resulting in expression of the GOI (Figure 1).

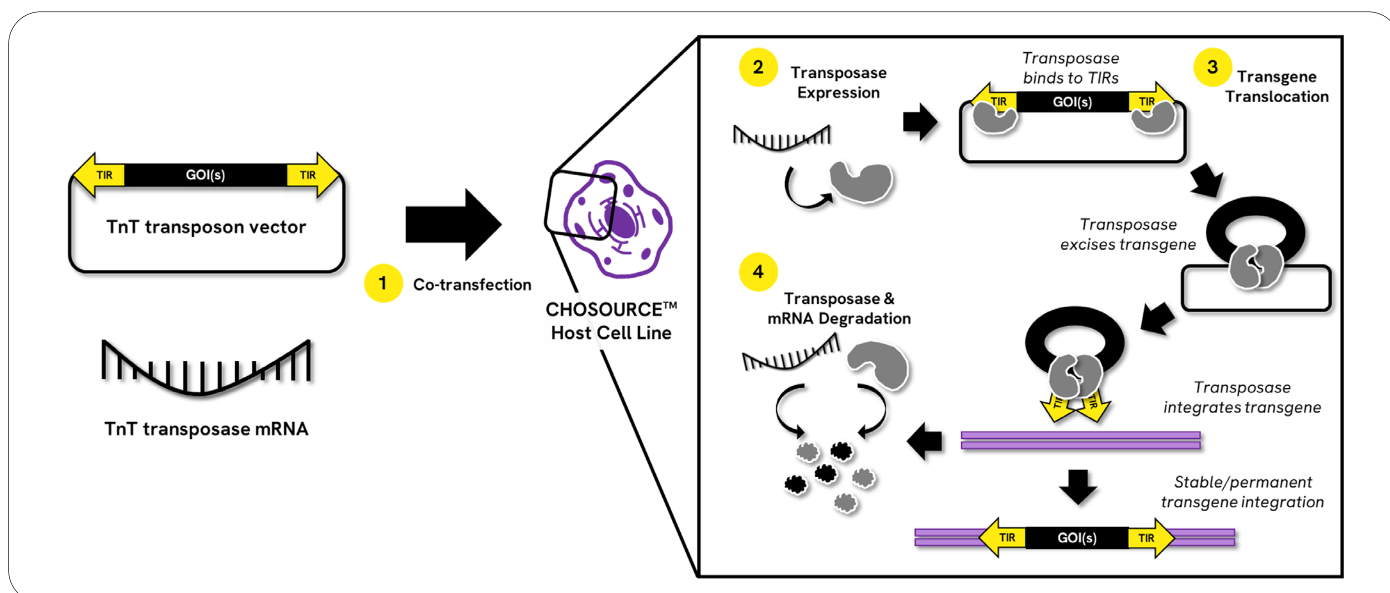


Figure 1: How does the CHOSOURCE TnT transposon technology work?

Expression of an IgG antibody in a CHOSOURCE host cell line typically involves the following steps:

- Molecular cloning to design and generate TnT vectors with the genes of interest
- Transfection of TnT vectors into a CHOSOURCE host cell line
- Selection of transfected cells with glutamine-free media
- Copy number variation analysis using droplet digital PCR (ddPCR) to assess the number of copies of the gene integrated into the genome
- A 14-day fed-batch study and titer assessment to determine pool productivity
- Product quality analysis to assess quality of the expressed molecule

Within Revvity's CLD pipeline, product quality is assessed using the LabChip™ GXII Touch™ system, which can assess an array of quality attributes such as N-glycan profile, charge variants, and impurities. The LabChip GXII Touch protein characterization system offers a high-throughput alternative to traditional methods (such as SDS-PAGE or CE-SDS) whilst providing quicker output as well as ease of use. Other methods such as mass spectrometry, require specialist knowledge to enable sample processing and data analysis. With LabChip, simple workflows are provided alongside the assays, to enable scientists to obtain high quality data without in-depth expertise.
A number of different assays are available for different requirements. For example, protein quality assay such as the ProteinEXact™ can be performed under reducing (quantification of antibody chains) or non-reducing conditions (to identify different conformations, glycosylation, homodimers, etc. of the antibody), and data can be analyzed via electropherogram, tabular, or virtual gel formats. This versatile instrument supports multiple assays for different applications, including protein characterization, nucleic acids, and beyond.

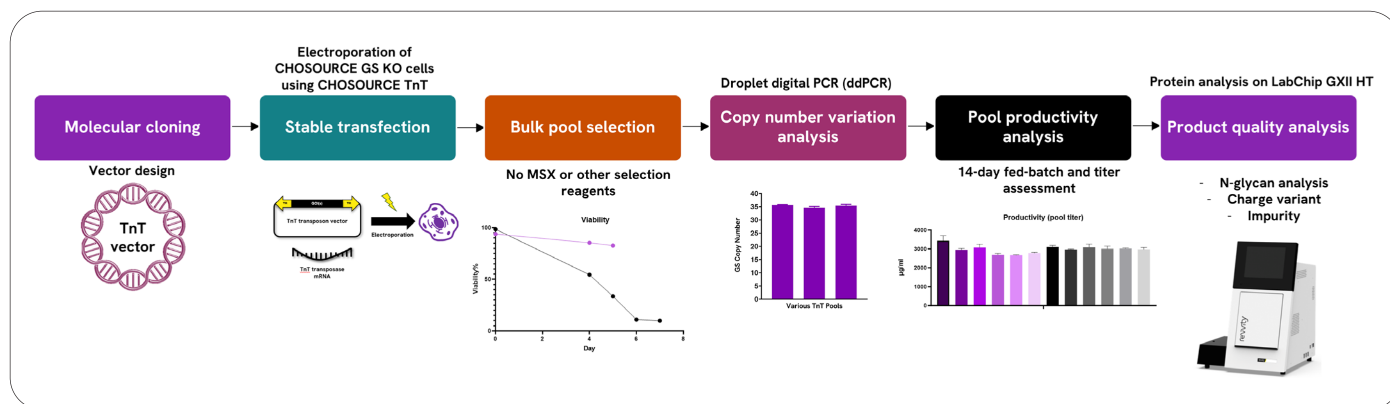


Figure 2: Antibody expression workflow with CHOSOURCE Expression Platform.

Assessing N-glycan profiles of antibodies expressed using the CHOSOURCE Expression Platform

N-linked glycosylation is a common post-translation modification on biotherapeutic antibodies, affecting efficacy, stability, pharmacokinetics, pharmacodynamics, and immunogenicity. Several variables can influence the glycosylation profile of antibodies, including the choice of media, cell line, and production methods.

At Revvity, LabChip is used for N-glycan analysis of antibodies expressed with the CHOSOURCE platform at pool as well as clone stages. In figure 3, the N-glycan profile of an IgG1 expressing pool and 5 monoclones isolated from this pool are shown. Supernatant from a 14-day fed-batch study of CHOSOURCE TnT cells expressing an IgG1 was protein-A purified and analyzed on the LabChip. The study demonstrated that all major glycans were present on the IgG1 molecule as expected and that the glycan profile was similar between the pool and its clones. Overall, this showed that the IgG1 expressed by utilizing the CHOSOURCE TnT technology displays the expected glycan profile, potentially allowing pool material to be used in early pre-clinical studies and accelerating CLD timelines.

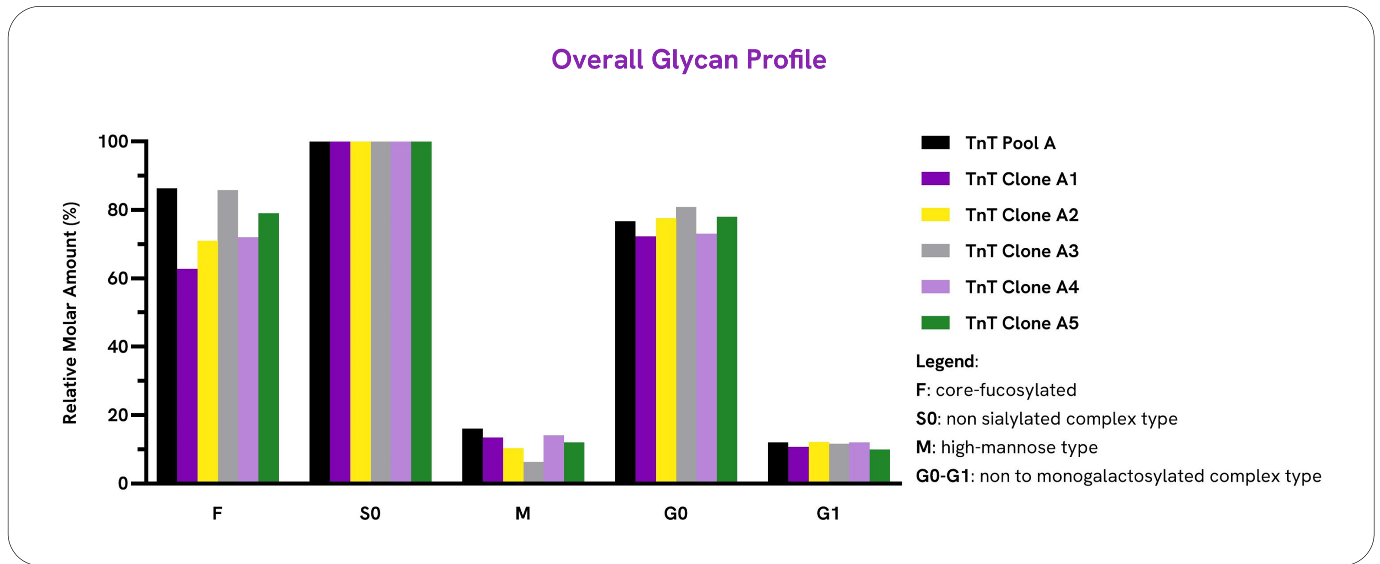


Figure 3: Glycan profile of CHOSOURCE-produced IgG antibodies.

Expression of bispecific antibodies with CHOSOURCE TnT technology

While biological antibodies are mono-specific with two antigen-binding sites that recognize the same epitope, technological advancements have resulted in heavily engineered, complex bispecific antibodies that can recognize two different antigens, allowing researchers to bridge tumor and immune cells for redirected cytotoxicity, block multiple targets to inhibit tumor growth, or enhance immune cell functionality[1]. A bispecific antibody can contain 3 - 4 chains with modifications that may result in incorrect combinations during expression.

CHOSOURCE GS KO cells were used to express a 4-chain asymmetric bispecific antibody with two heavy and light chains each. And while the 'knob-into-holes' modifications preclude the development of several incorrect combinations, homodimers can still occur, resulting in knob/knob or hole/hole bispecific antibodies (Figure 4). These homodimers (146.76 or 146.97 kDa) have a very similar molecular weight to the desired heterodimer (147.18 kDa), making it nearly impossible to separate out these contaminants during downstream processing steps.

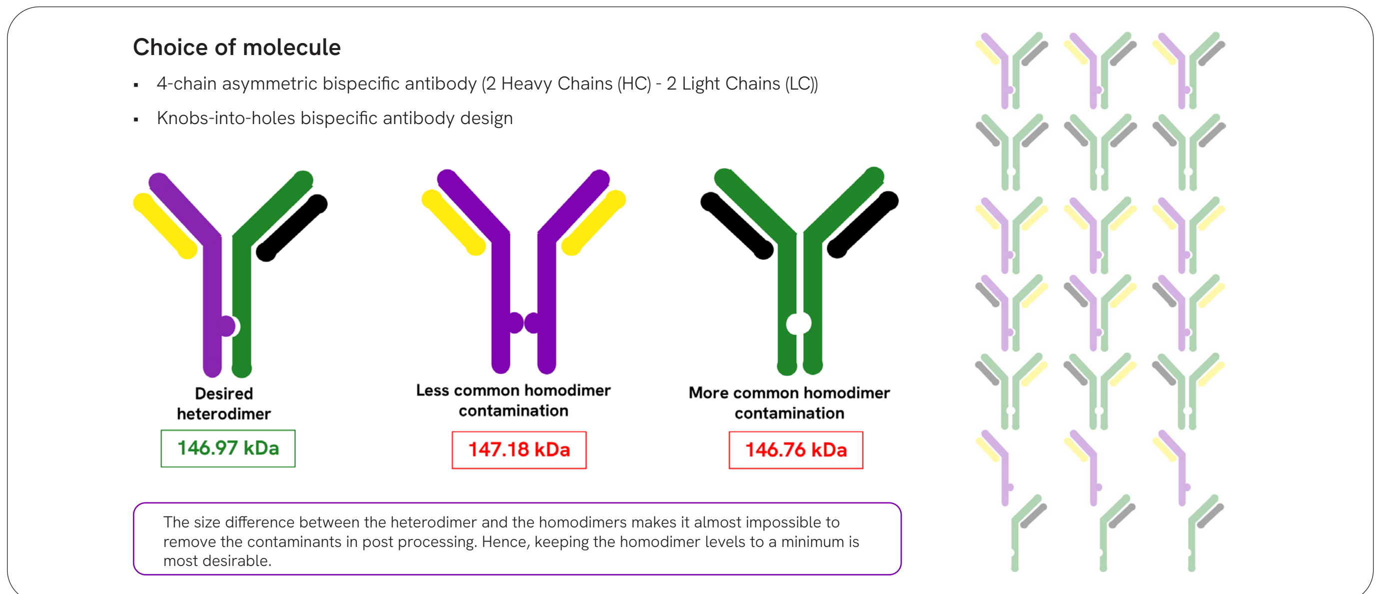
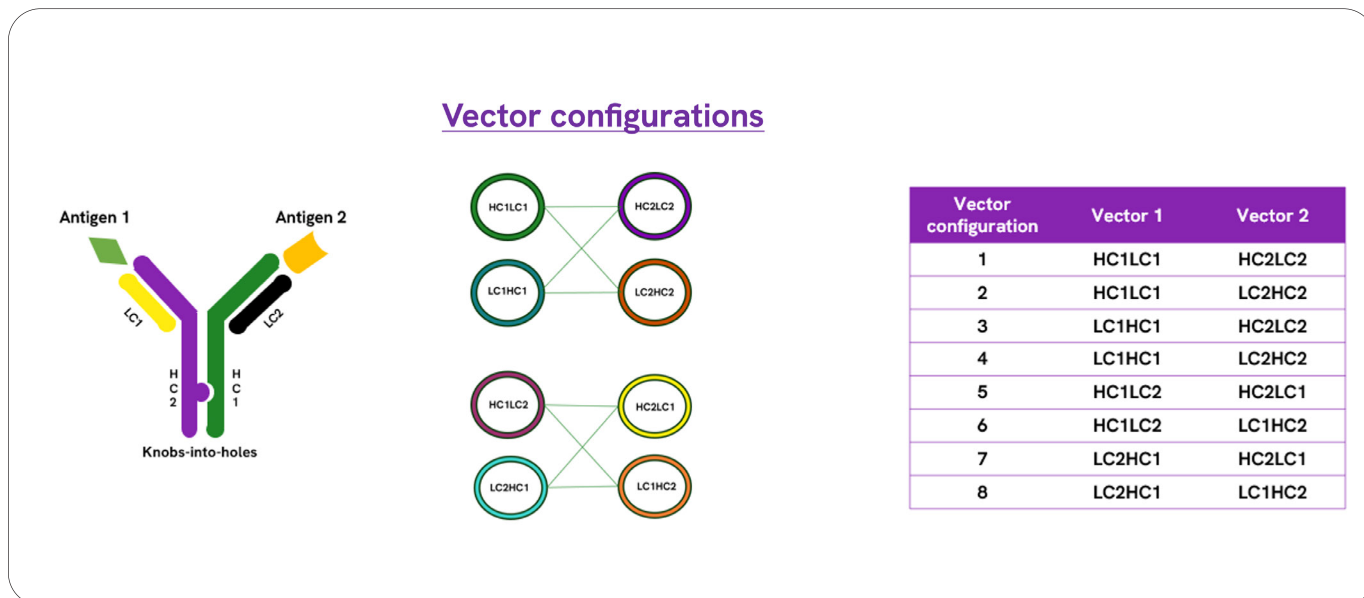


Figure 4: Incorrect chain combinations for bispecific antibodies.

For bispecific antibody expression using the CHOSOURCE expression platform, the four chains are cloned into two different vectors and transfected into CHOSOURCE GS KO cells. For this set of experiments, a total of four chains were cloned into 8 different vectors in order to test different configurations as shown in figure 5. The subsequent steps are as outlined in the antibody expression workflow in figure 2.



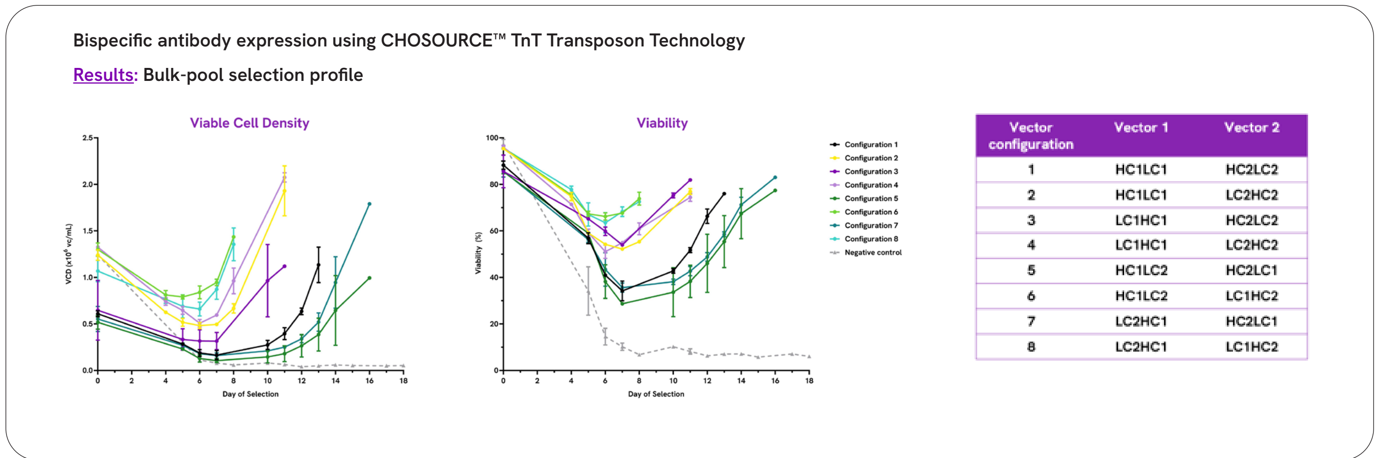
Vector configuration	Vector 1	Vector 2
1	HC1LC1	HC2LC2
2	HC1LC1	LC2HC2
3	LC1HC1	HC2LC2
4	LC1HC1	LC2HC2
5	HC1LC2	HC2LC1
6	HC1LC2	LC1HC2
7	LC2HC1	HC2LC1
8	LC2HC1	LC1HC2

Figure 5: Vector configurations for bispecific antibody chains.

Bispecific antibody characterization

To generate pools expressing all four chains of the bispecific antibody, the vectors were co-transfected into the GS KO host cell line in pairs, in a 1:1 ratio (vector 1:vector 2) along with CHOSOURCE TnT transposon mRNA. Two pools were generated for each co-transfection. After undergoing selection, the pools were recovered based on the vector configuration anywhere from 8 - 16 days. This shows that the antibody chain position in each vector impacted the pools' selection recovery.

Copy number analysis for each pool was performed to determine the number of integrated copies of the antibody chains. As expected, heavy and light chains cloned into the same vector were integrated in a 1:1 ratio with the CHOUSOURCE TnT transposon technology. As vectors were also transfected at a 1:1 ratio, the expectation was that all four chains would be incorporated in equal numbers, but this was not the case (e.g. configurations 1-3, 5, 6). This may be due to the incorporation of knob-knob homodimers that aggregated and caused cell death, meaning cells that expressed more knob-heavy chains may not have survived selection post-transfection. The GS copy number was incorporated at a higher number, closely matching the expected sum of average copy number of the integrated chains since GS was incorporated into both vectors (Figure 6).



Vector configuration	Vector 1	Vector 2
1	HC1LC1	HC2LC2
2	HC1LC1	LC2HC2
3	LC1HC1	HC2LC2
4	LC1HC1	LC2HC2
5	HC1LC2	HC2LC1
6	HC1LC2	LC1HC2
7	LC2HC1	HC2LC1
8	LC2HC1	LC1HC2

Figure 6: Bulk-pool selection profile of bispecific antibodies.

Antibodies produced by all pools were analyzed in non-reduced conditions on LabChip GXII Touch, allowing them to be separated by size while being compared against the reference bispecific antibody. This enables the identification of different forms of the antibody such as heterodimers, homodimers, glycosylated variants, and free chains. Configuration 8 displayed the profile that most closely matched the reference standard (Figure 7).

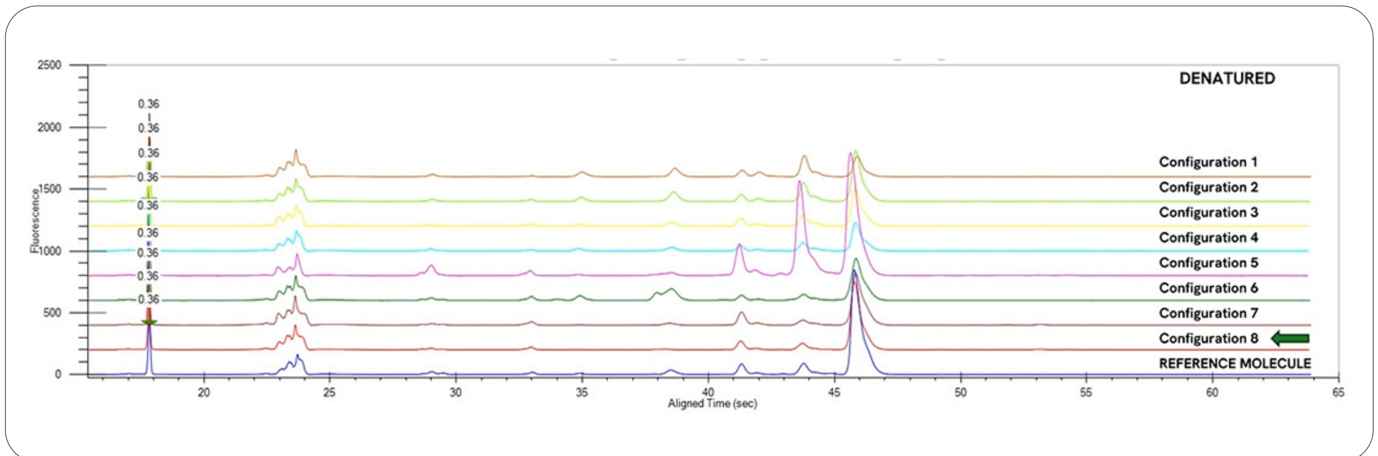


Figure 7: LabChip analysis of non-reduced bispecific antibody configurations.

Finally, analysis was performed using reduced samples. This helped identify the individual heavy and light chains of the antibody. It was noted that although integration of all chains occurred in a similar ratio in the pools for configurations 4 and 7, the ratio of chain expression was not the same. On the contrary, configuration 8 demonstrated 94% correct confirmations, most closely matching the profile of the reference standard. This LabChip assessment allowed the scientists to identify the most promising pool early in their cell line development process, saving time and resources. Revvity's workflow enables and accelerates critical phases of cell line development.

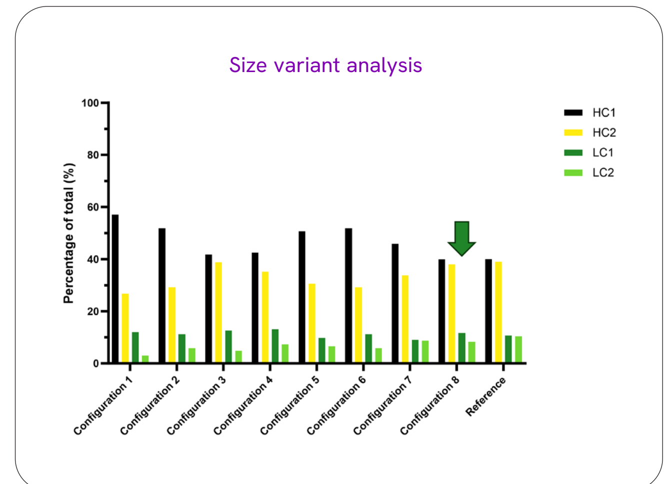


Figure 8: LabChip analysis of reduced bispecific antibody configurations.

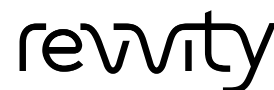
Conclusion

Biotherapeutic development requires extensive monitoring and testing to maintain product quality throughout the cell line development process. Being able to identify incorrect quality profiles early on during CLD can be cost-effective and time-saving for researchers. Advanced expression platforms, such as Revvity's CHOSOURCE Expression Platform, can reduce timelines during CLD and can produce biologics with required critical quality attributes. In addition, analytical platforms, such as the LabChip GXII Touch from Revvity, offer methods to characterize protein quality. When implemented early in the process, reliable and high-throughput methods such as the LabChip can potentially fast-track the CLD process.

References

1. Liguori, Luigi et al. "Bispecific Antibodies: A Novel Approach for the Treatment of Solid Tumors." Pharmaceutics vol. 14,11 2442. 11 Nov. 2022, doi:10.3390/pharmaceutics14112442

The CHOSOURCE™ Platform is available for research, clinical, diagnostic, and commercialization applications under specific licenses from Revvity. The platform is also available for services under a service license from Revvity.



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