ABZENA

Enabling better biopharmaceuticals

Biosimilar Development
in CHO, NS0 & Sp2/0 with enhanced PQA assessment

Abzena offers CHO, NS0 and Sp2/0 biosimilar cell line development programmes with bespoke quality assessment tailored to individual projects.

- High transcription vector system
- Vector components fully sequenced and traceable
- Highly efficient transfection process
- Fully traceable host lines (CHO, NS0, Sp2/0 and others)
- Host cell lines certified sterile and mycoplasma-free
- Final optimised cell lines ready for transfer to cGMP manufacturing facility

Background

An important decision in the development of a biosimilar is the choice of cell line. Changing from the originator cell line to an alternative cell line can lead to changes in post translational modification and deviation from the originator Product Quality Attributes (PQA). These changes can affect the characteristics of the final product (Table 1). In particular, variations in glycosylation patterns are known to affect antibody-dependent cell cytotoxicity (ADCC), half-life, stability, folding and immunogenicity.

Developing biosimilars

Maintaining biosimilarity is critical for regulatory approval and Abzena can help you achieve this by developing your biosimilar cell line using the originator cell type. We have CHO, NS0, and Sp2/0 cell lines adapted to serum-free growth available for development programmes. During development of the cell line we assess a range of critical product quality attributes (PQA) to check consistency of these parameters with the desired product.

Glycoforms	Impact
Afucosylated variants	ADCC activity
High mannose-containing oligosaccharides	Immunogenicity
Mono-galactosylated (G1F) forms	PK properties
Di-galactosylated (G2F) forms	PK properties
Sialyic acid	Binding, immunogenicity

Table 1. Small differences in glycosylation patterns have been shown to have significant effect on specific biopharmaceutical properties.

Delivering on expectations

Yield and product quality are key to biosimilar development and Abzena has developed technologies, including Composite CHO™ and pANT™ vectors, to achieve the high expression levels of the desired antibodies and proteins (Figure 1). We ensure all materials we use to develop the cell line are free of animal-derived products and the cells are grown in a chemically defined medium. We are experienced in the transfer of cell lines to cGMP manufacturing facilities to expedite scale-up.

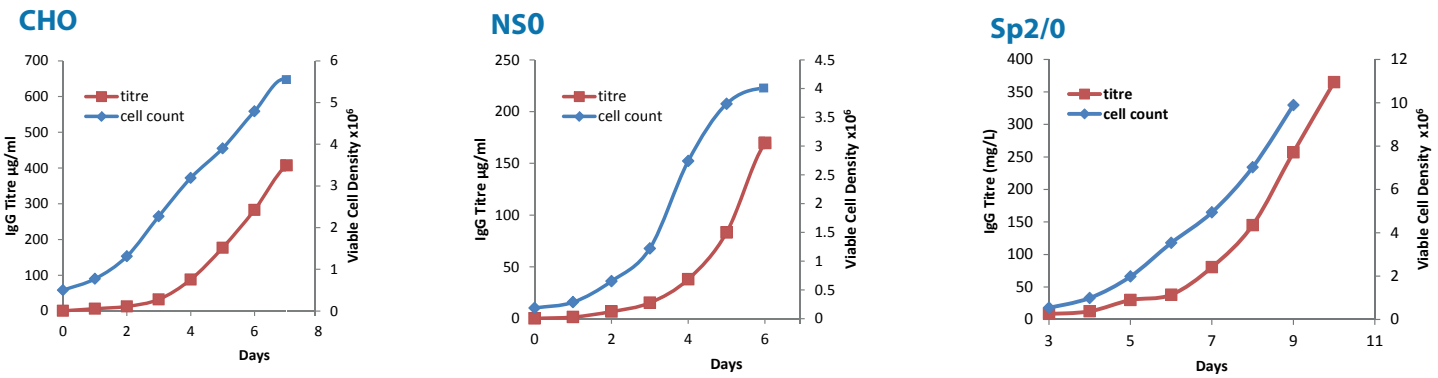

Figure 1. Titre and cell count for 50, 70 and 30ml batch cultures of CHO, NS0 and Sp2/0 cell lines respectively.

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pANT™ vector

pANT™ maximises recombinant protein expression through the use of a proprietary UTR (Untranslated Terminal Region) to enhance protein production, and a modified *dhfr* gene for the rapid amplification of vector copy numbers in the cells.

Proprietary vector:

- Modified 5' UTRs for increased protein expression
- DHFR selection allows industry standard MTX amplification
- Driven by a strong CMV I/E promoter

For recombinant antibodies:

- Contains both heavy and light chain genes in a single vector
- Constant regions contained on genomic fragments (with introns) for increased expression levels
- Alternative IgG sub-classes (IgG1, IgG2, IgG4, hinge-modified (S241P), enhanced FcR binding)

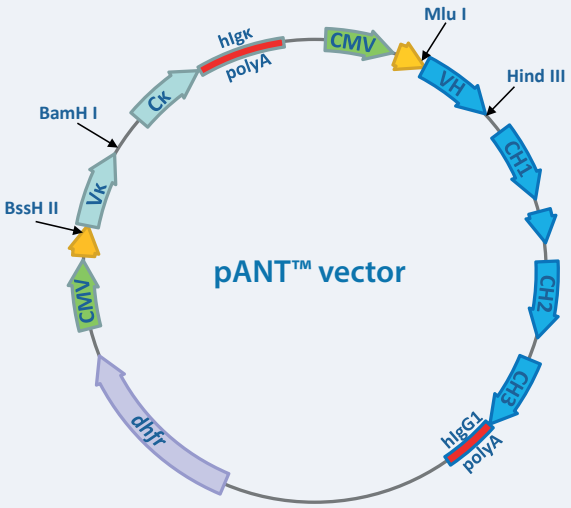


Figure 2. Visualisation of a dual-chain pANT™ vector designed for recombinant antibodies. Yellow arrows indicate modified 5' UTRs.

Product quality attributes (PQA) assessment

To ensure consistency during the generation of manufacturing cell lines we have an approach whereby cell line and product quality are assessed at multiple stages, as an integral part of the cell line development process.

Product aggregation

- Analysis of product aggregation, subunit dissociation or degradation
- Intact mass analysis
- Assessment of sample purity and suitability of purification method

Product integrity

- Confirmation of full protein and subunit mass
- Confirmation of subunit dissociation
- Analysis for loss of subunit or fusion partner
- Analysis of potential degradation
- Confirmation of biosimilar concentrations

Quality Control (QC) testing

- Full gene sequencing including full expression cassettes
- Growth and productivity assessment
- CFR21 compliance: mycoplasma, bacterial, fungal & viral sterility testing
- Cell line identity test
- Research cell bank thaw testing and productivity assessment
- Cell line stability ensures productivity maintained over generations

Product glycan profiling

- N-glycan peak identification
- Monosaccharide analysis
- Sialic acid analysis
- Fucosylation
- Matching of specific glycan profile to innovator product

Product activity

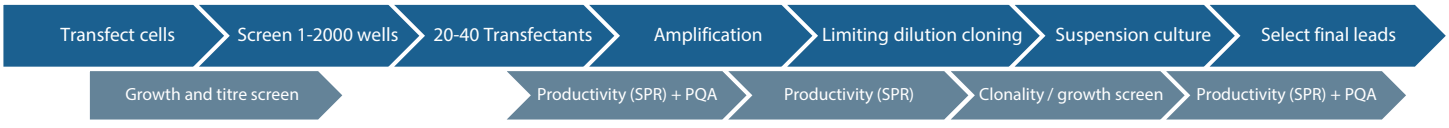
- Confirmation of binding characteristics
- Confirmation of activity/functionality
- Assessment of effector functions (ADCC/CDC) for antibodies

Facilities

Abzena ensures minimal risk for cross-contamination through dedication of specific equipment to individual projects and through routine sterilisation and servicing. Cell lines are stored under liquid nitrogen using facilities that offer 24-hour security, power and liquid nitrogen backup.

Timeline

Abzena's biosimilar cell line development projects take 24 to 36 weeks and include extensive PQA testing throughout.



Working with Abzena

Abzena's services are tailored for each project to ensure that the objectives are met or exceeded. Experienced and dedicated project teams are assigned to each study to focus on progressing projects from design to results in the minimum amount of time. Our clients uniformly judge us as professional and attentive partners who deliver quality results in a timely manner.