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Aragen protein science solutions: Industry leading upstream and downstream capabilities

The production of biopharmaceuticals typically begins in living cells thorough the upstream process followed by purification in the downstream process. As a first step, in the upstream process the mammalian or microbial cells are engineered to produce a specific biologic in milliliter quantities. In process development phase the transfected cells are cultivated in controlled environment within a bioreactor to produce several thousand liters of the culture so that the desired biopharmaceutical can be manufactured in greater Kilogram quantities. The generation of the biopharmaceutical is followed by its isolation and purification by removing mixture of host cell proteins, cell debris, nutrients, and waste materials by separating cells from the media. In mammalian cultures the protein is secreted into the media and hence the supernatant is collected for further purification. In microbial production, the protein can accumulate within the cells, therefore the cells are first disrupted, and then the cell debris are separated from the protein of interest. Insoluble products including unfolded, pure proteins stick together. They are known as inclusion bodies that are refolded to achieve their native and active conformation.

Aragen life sciences is a leading service provider to produce range of biopharmaceuticals. With more than two decades of experience in the domain, we provide end-to-end upstream and downstream solutions. Further, our expert scientific personnel work in advanced state-of-the-art laboratories to offer custom solutions for expressing wide range of biologics, efficient process development, high throughput analytics, purification, and a fast and secure supply of material throughout the life cycle of a given biopharmaceutical. Aragen considers all the clinical aspects anticipated during the biopharmaceutical development. We ensure in the beginning that an upstream process development project is scalable, compliant, and economical for large-scale cGMP manufacturing. We focus on producing the product that meets quality and regulatory requirements relative to safety, purity, potency, identity, and immunogenicity through rigorous characterization.

Comprehensive services for biopharmaceutical production:

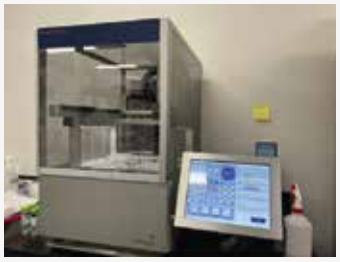
Molecular Biology services:

Custom gene Synthesis and codon optimization:

Aragen provides customized gene synthesis services. We synthesize the DNA sequences chemically according to the customer request that includes the DNA sequences that is difficult to clone, including repeated sequence, GC rich sequences, hairpin structure, same nucleotide repeated sequence etc. We guarantee 100% sequence accuracy and free codon optimization service to save project time.

Expression vector design and synthesis:

Aragen understands the unique requirement of each client. Our molecular biology experts help the client to construct a completely customized vector. Using our de novo DNA synthesis capability, we will make any fragments that are required for the vector. Our know-how and experience help us provide premium quality molecular biology and protein expression services to clients from biotech and pharmaceutical companies.

Upstream development (Protein production services):

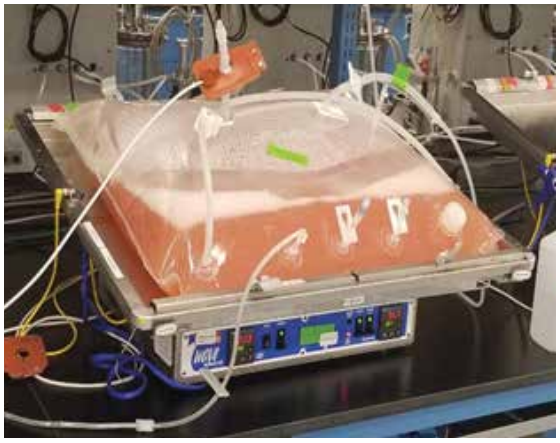
Identifying a strong host cell line platform is a critical factor to produce an efficient biologic. Several factors are to be considered before selecting the host cell line, such as particular project requirements and the biologic being anticipated. Aragen will assist its customers with constant interactions during the early stages by selecting the host cell line after considering all the physical and chemical properties of the protein. Some of our host cell line platforms are CHO-DG44, CHO-GS, HEK293, SP2/0, and NS0. These platforms are very popular among our clients for expressing a broad range of molecules, including antibodies, bi-specifics, and fusion proteins. Aragen's Cell Line Development (CLD) expertise using a royalty-free and regulatory-friendly platform, makes Aragen your partner of choice.

Aragen has full capabilities in transient and stable expression for both small and large scales. Our expression systems and scales are described in tables below.

Protein Production: Transient Transfection	
Transfection system	**Lipid Base**
Cell Type	HEK293, CHO-S, Expi-CHO, Expi-HEK293
Scale	30mL to 10L in Shaker Flask
	5L to 20L in Wave
Capacity	20 Construct at a time
Dedicated E. Coli Lab.	Capacity up to 10L production with purification

Protein Production: Stable Cell Line
Production run size from small scale ~2L to max 240L in Wave.
Expertise in CHO, CHO-GS, CHO-S, Hybridoma.
Production using Aragen's STD process as well as customized Client's process.
Expertise in controlled Stirred Tank Bioreactor- 12X1L, 3X5L and 1X10L Bioreactor.





Downstream development (Protein purification services):

Aragen has established downstream platforms to help our customers to move quickly into products manufacturing phase. Our decade of experience had enabled us to offer customized and innovative solutions to meet the customers' growing needs. We have optimized our downstream processes to remove impurities and increase yields with minimal steps and optimal recovery, resulting in more cost-efficient manufacturing process. Our analytical teams are fully integrated to ensure the product quality attributes. Our downstream development capabilities and Analytical capabilities are listed below.

- Downstream: Protein purification
  - Protein A/G, IEX, HIC, Ni-NTA, etc.
  - Process design with scouting studies to identify resin, buffers, and elution conditions
  - High purity: low endotoxin, low host cell proteins, and DNA
- Capacity-
  - Handle 20 small scale samples at a time with a turnaround time of less than a week.
  - Up to 20L one-step purification with a one-week turnaround.

Protein Analytics	Quality Attribute	Platform
Protein concentration by A280	Quantity	UV-Vis
A280, A320, Appearance	General appearance- Turbidity	
Free Thiol by Ellman's Assay	Primary Structure- Free Thiol	
Enzyme Kinetics (Kcat, Kcat/Km)	Potency	
N-Glycan profiling by CE	Primary Structure - PTMs	SCIEX PA 800Plus
Charge variants by cIEF	Purity-charge variants	
Charge variants by CZE	Purity-charge variants	
Size variants by (Fragments) by CE-SDS	Purity Size varients	
LC-MS intact Mass (non-reduced, reduced, +/-PNGaseF)	Primary structure-MW	LC-MS/MS Agilent QTOF attached to nanoflow Chip-Cube or microflow UPLC (ESI)
LC-MS/MS PepMap	Primary structure-PTMs, Modifications	
PepMap UV	Identity	
N-Glycan profiling by RP-MS or HILIC-MS	Primary structure-PTMs	
Glycation by LC-MS/MS	Primary structure- Modifications	
Absolute mass of monomer and aggregates	MW-Size variants	SEC-MALS (Wyatt 18-angle MALS)
Tm (Fluorescence), Tagg (SLS), Size and PDI (DLS)	Thermal stability, Higher order structure (HOS)	Unchained Labs UNcleTM
Slab gel IEF	Purity - Charge variants	Gel Electrophoresis
SDS Page (non-reduced and reduced)	Purity – Size variants	
Western blot	Identity	
Binding Kinetics (Kon, Koff, KD)	Potency, Identity	BioLayer Interferometry (BLI) ForteBio Octet RED Or Surface Plasmon Resonance (SPR) Biacore
FcR and C1Q binding	Half-life , Safety	
Protein concentration, antigen binding titer	Quantity	
Epitope Binning	Epitope Characterization	
Functional blocking	Potency, Identity	
Yes/No binding	Identity	
Binding ELISA	Potency	Molecular Devices SpectraMax
HCP ELISA (Cygnus)	Safety	
Residual Protein A/G/L by ELISA	Safety	
Endotoxin by Limulus Amebocyte Lysate (LAL)	Safety	Charles River Endosafe
Bioburden, Sterility Test	Safety	Culturing