CHOin1™

Serum Free Medium and Feed

Clonesecurity

Clone specific customization
Media & feed

Spent media analysis

Systematic experimentation &
Comprehensive analysis

HiMedia Laboratories Pvt. Ltd
Animal Cell Culture
Research & Development

* The data present in this dossier is obtained as a part of the project sponsored by DBT-BIRAC under National Biopharma Mission Programme

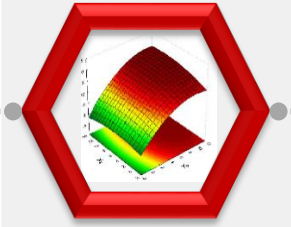
INDEX

01

CLONE DEVELOPMENT

- Transfection
- Expression
- Clonal selection

02



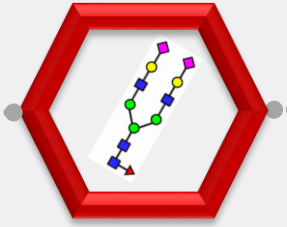
MEDIA DEVELOPMENT

Step wise approach for clone specific media and feed development by DOE at different scales

From shake flasks to Bioreactors

- Screening by Plackett Burman
- Interaction analysis by factorial design
- Optimization by response surface method
- Feed development
- Feeding strategy

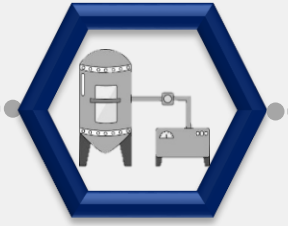
03



QUALITY CONTROL

- Cell sustenance
- IgG Titer
- Specific productivity
- Charge variants
- Aggregation pattern
- Glycosylation pattern

04



UPSTREAM PROCESSING

05

DOWNSTREAM PROCESSING

We are there to assist you at every step

MEDIA & FEEDS

SERUM FREE MEDIUM

CHOin1™
Serum Free CHO Medium
Animal Component Free, Chemically Defined
With Pluronic F-68®
Without L-Glutamine and Sodium bicarbonate

SFM007AP-10L
SFM007AP-50L

UNIVERSAL FEED SUPPLEMENT

CHOin1™ Feed Medium
Chemically Defined, Animal component-free
With D-Glucose and Pluronic F68®
Without L-Glutamine and Phenol red

SFF006P-1L
SFF006P-5L

EVALUATED ON

Clone type	Product	Disease treated	Mechanism of action
CHO-GS	Trastuzumab	HER2-positive breast cancer	Trastuzumab works by attaching itself to the HER2 receptors on the surface of breast cancer cells and blocking them from receiving growth signals. By blocking the signals, Trastuzumab can slow or stop the growth of the breast cancer. Trastuzumab is an example of an immune targeted therapy. In addition to blocking HER2 receptors, Trastuzumab can also help fight breast cancer by alerting the immune system to destroy cancer cells onto which it is attached
CHO-GS	Bevacizumab	Brain tumor and cancers of the kidney, colon, rectum, lung, or breast	Bevacizumab targets VEGF-A, an isoform of VEGF that stimulates endothelial cell proliferation and subsequent migration. Bevacizumab specifically binds to the VEGF-A protein, thereby inhibiting the process of angiogenesis
CHO-DHFR	Adalimumab	Rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, inflammatory bowel disease, skin and joint manifestations of psoriasis.	Adalimumab specifically targets and blocks TNF-alpha which contributes to the inflammation that causes the abscesses, inflammatory nodules.

SYSTEMATIC SCALE UP APPROACH

Scale I

Scale I:
High throughput screening (10 – 15ml)

01

Accuracy by Automization
Screening of >1000 media combinations in Bioreactor tubes

Scale II

Scale II:
Growth Factor Optimization (50ml)

02

Top Down Approach
Screening of >500 media combinations in shake flasks to identify critical growth factors

Scale III

Scale III:
Lab scale bioreactors (0.4L- 1L)

03

Process Optimization
Process control at Lab scale bioreactors for productivity

Scale IV

Scale IV:
Complete Process Optimization (10L)

04

Process Validation
Suitability analysis for quantitative and qualitative productivity of the process for commercial application

Optimized Medium and Feed...
Assurance of high productivity

TRASTUZUMAB

CLONE

PERFORMANCE CHARACTERISTICS

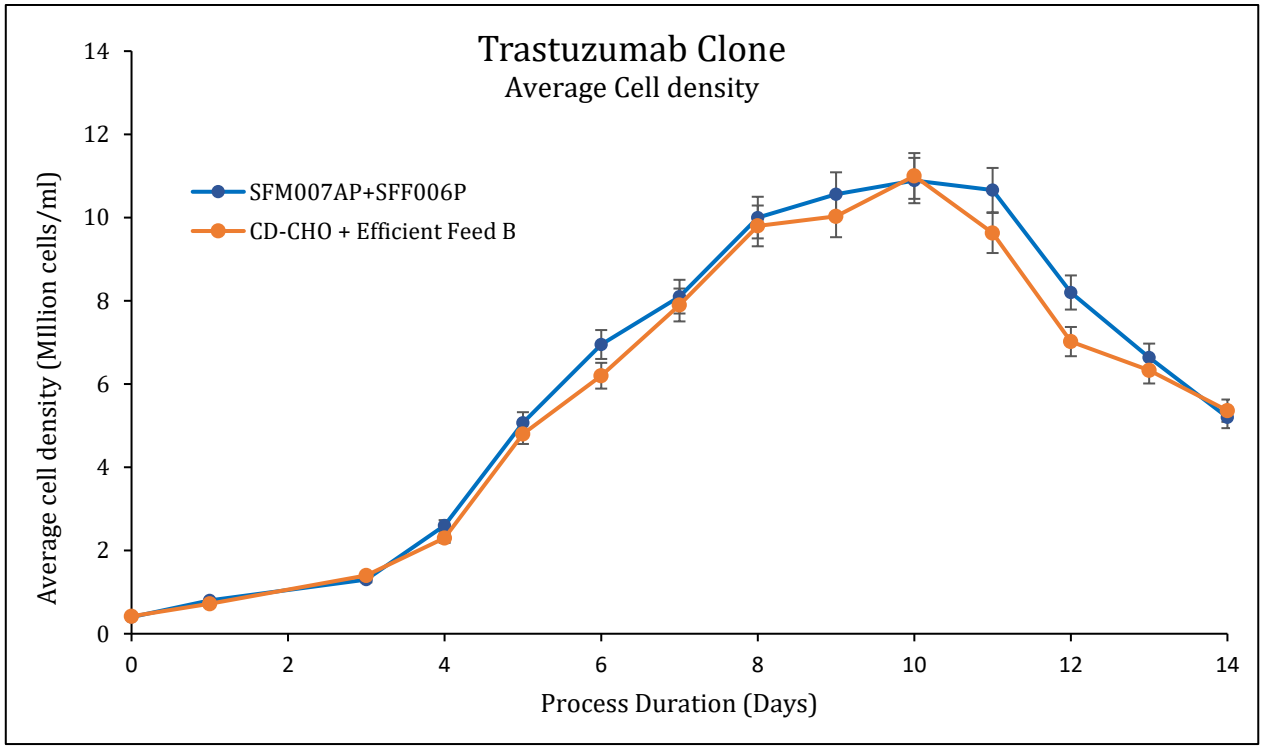
Trastuzumab producing CHO clone

Experimental conditions

Culture parameters	Details
Culture Vessel	: Scale up from Bioreactor tubes (50ml) to Sartorius B-Twin **10L Bioreactors** Data represented here is for Sartorius B-Twin **10L Bioreactors**
Process type	: Fed-batch culture
Combination details	1. Medium SFM007AP + Feed (SFF006P) 2. CD-CHO (Thermo) + Efficient Feed B (Thermo)
Cell line	: CHO clone producing mAb Trastuzumab
Culture conditions	: 80rpm, 37°C
Instruments used	1. Sartorius B-Twin 10L Bioreactors 2. Biochemical analyzer (Nova Biomedical) 3. HPLC (IgG titer estimation & characterization)
No. of vessels per combination	: 1
Seeding density	: 0.5 million cells per ml
Seeding viability	: 96.33%
Duration of experiment	: 12 - 15 days
Test parameters and frequency	1. Cell density: Daily 2. Cell viability: Daily 3. IVCC: Daily 4. IgG Titer: Alternate day after Day 8 5. IgG Characterization: Harvested sample 6. IgG potency testing and functional assays

Cell density (Million cells/ml)

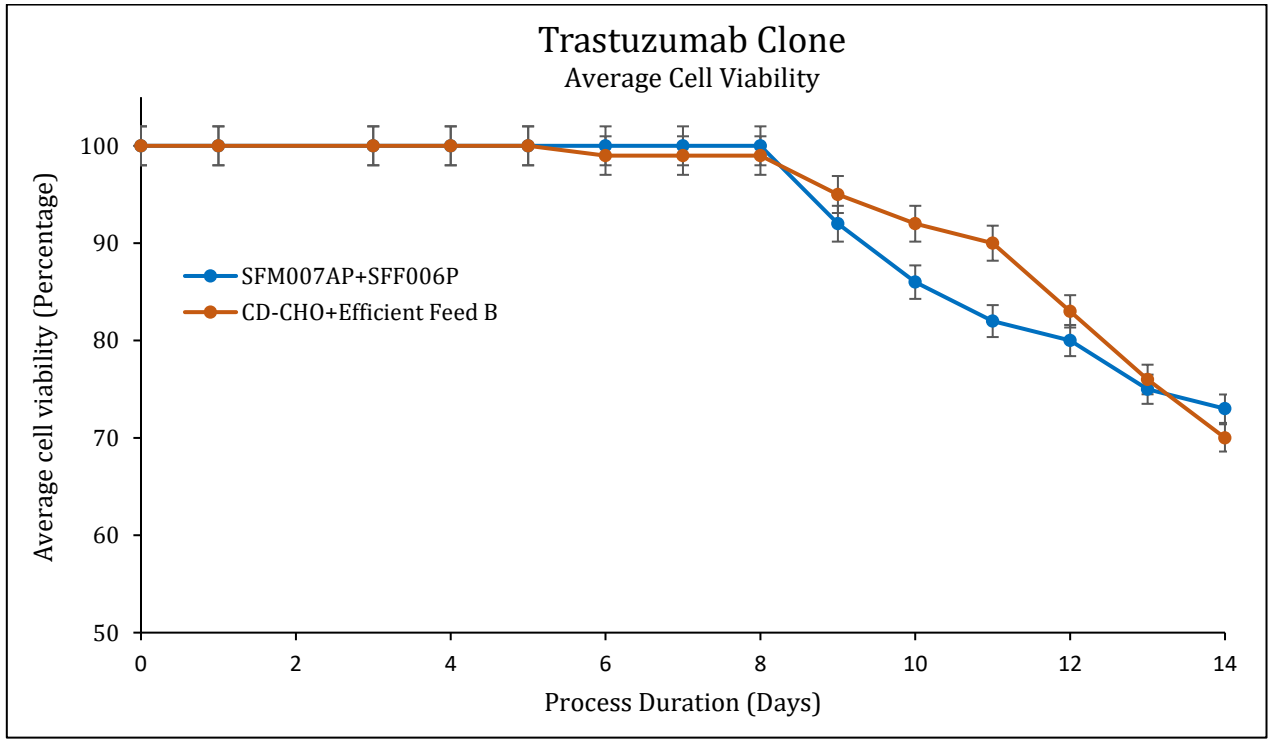
Combination	Incubation time (Day)													
	0	1	3	4	5	6	7	8	9	10	11	12	13	14
SFM007AP + SFF006P	0.40	0.80	1.30	2.60	5.07	6.95	8.10	10.00	10.56	10.89	10.66	8.20	6.64	5.20
CD-CHO+ Feed	0.42	0.72	1.40	2.30	4.80	6.20	7.90	9.80	10.03	11.00	9.63	7.02	6.33	5.36



Peak density: 10 to 11 million cells/ml

Cell viability (Percentage)

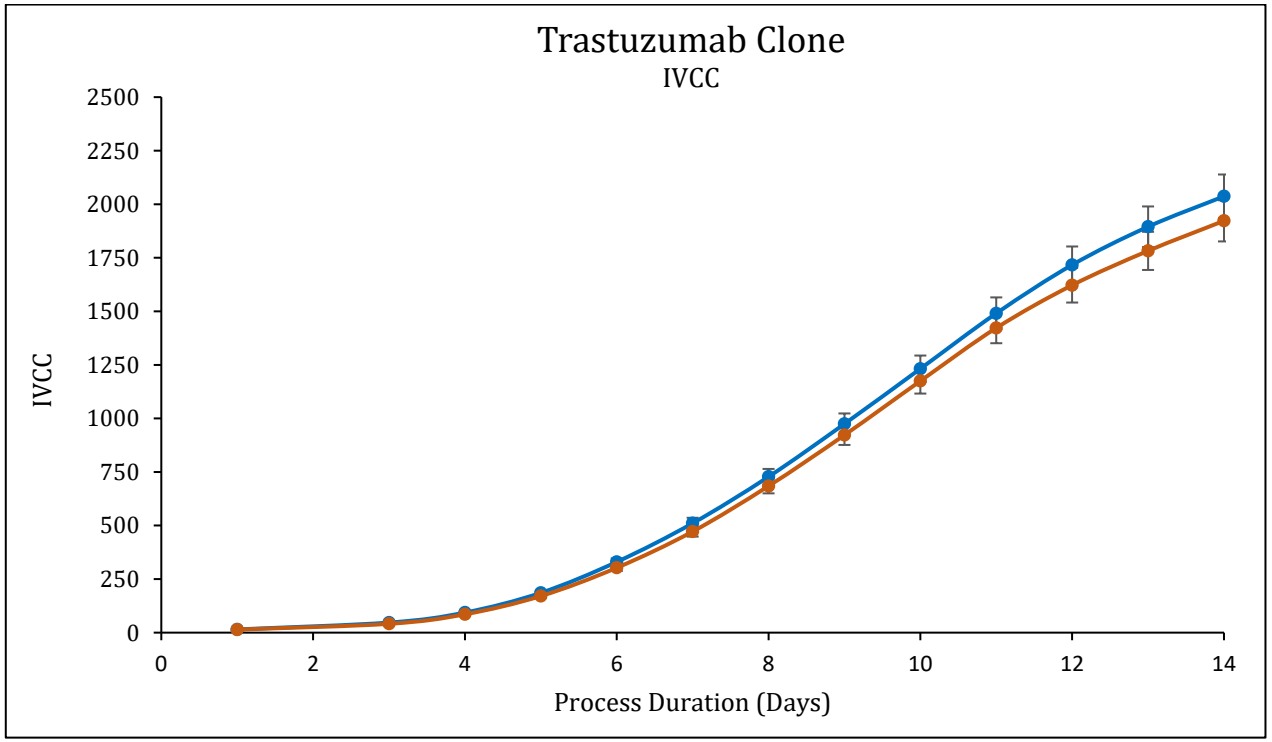
Combination	Incubation time (Day)													
	0	1	3	4	5	6	7	8	9	10	11	12	13	14
SFM007AP + SFF006P	100	100	100	100	100	100	100	100	92	86	82	80	75	73
CD-CHO+ Feed	100	100	100	100	100	99	99	99	95	92	90	83	76	70



Sustained Cell Viability till Day 12

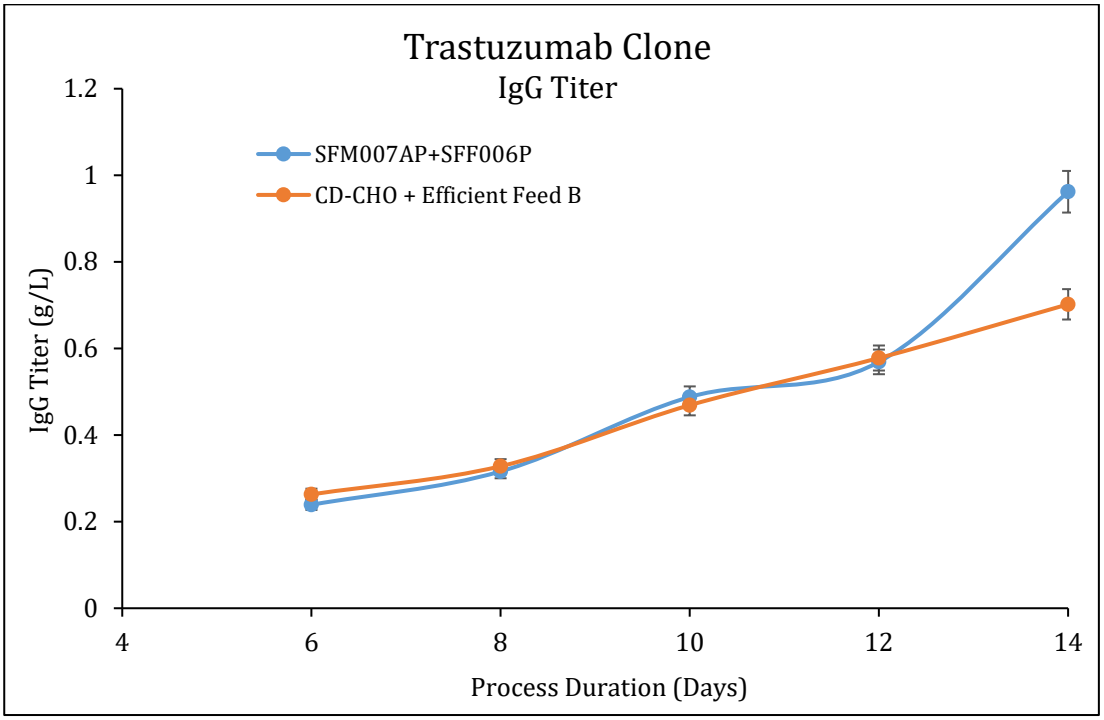
IVCC

Combination	Incubation time (Day)												
	1	3	4	5	6	7	8	9	10	11	12	13	14
SFM007AP + SFF006P	14.8	47	93.8	185.84	330.08	510.68	727.88	974.6	1232	1490.6	1716.92	1895	2037.08
CD-CHO+ Feed	14.1	41.24	85.64	170.84	302.84	472.04	684.44	922.4	1174.76	1422.32	1622.12	1782.32	1922.6



IgG Titer

Combination	Incubation time (Day)				
	6	8	10	12	14
SFM007L + SFF001P	0.239	0.316	0.488	0.569	0.962
CD-CHO+ Feed	0.263	0.328	0.469	0.578	0.702



PERFORMANCE SUMMARY

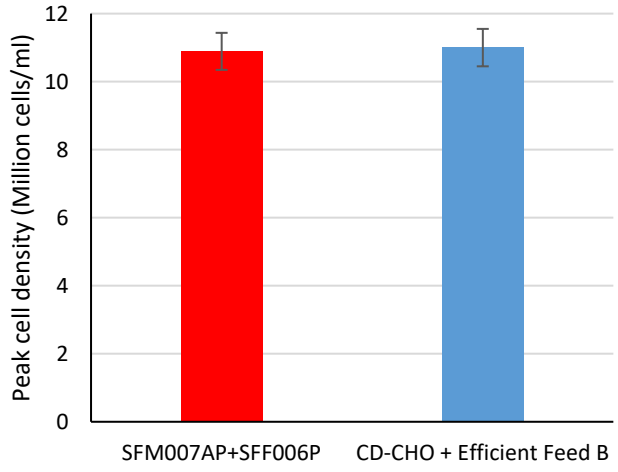
Trastuzumab producing CHO clone

Cultural Characteristics

Test parameter	HiMedia SFM007AP + SFF006P	Thermo CD-CHO + Efficient Feed B
Peak cell density	10 – 11 million cells/ml	10 – 11 million cells/ml
Peak cell viability	More than 80%	More than 80%
IgG titer	0.962g/L	0.702g/L
Specific productivity	5.3 pg/ cell / day	5.7 pg/ cell / day

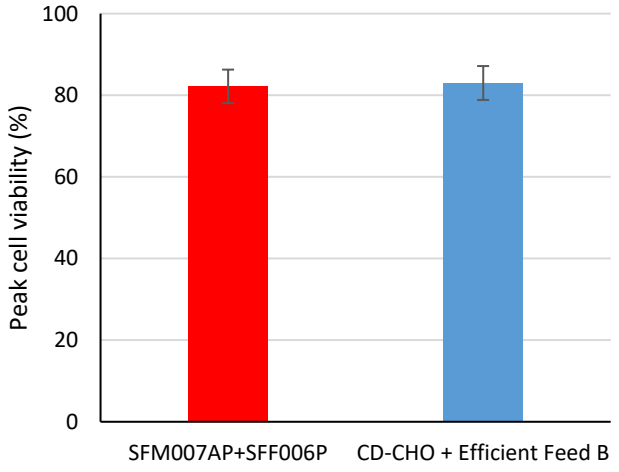
Peak Cell Density

10 – 11 million cells/ml on Day 10



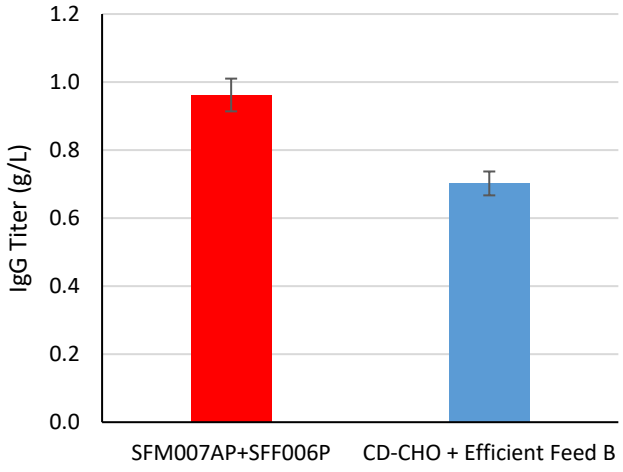
Cell Viability

More than 80% viability up to Day 12



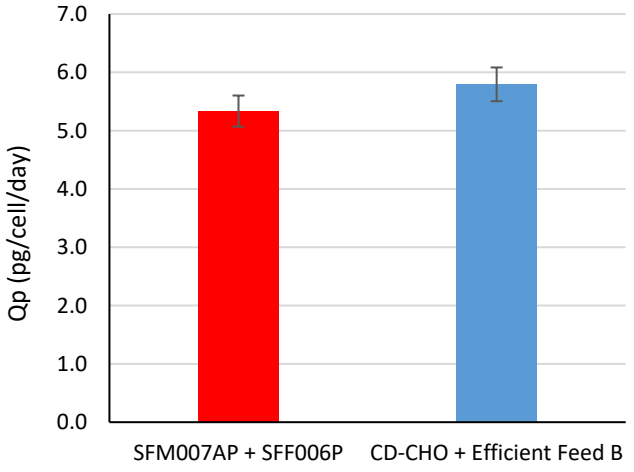
IgG Titer

~ 1g/L IgG Titer on Day 14



Specific Productivity

Qp > 5 pg per cell per day



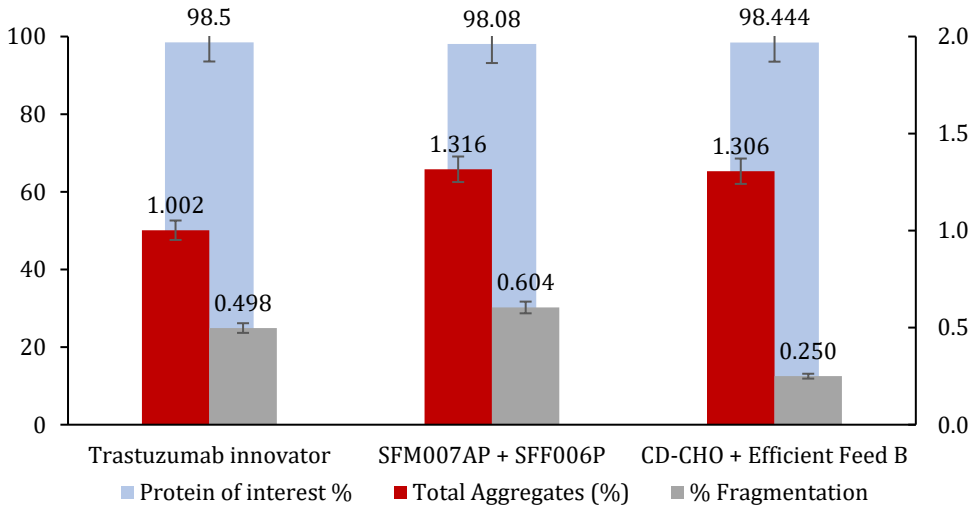
❖ Data represented over here is of 10L Bioreactor scale level

Trastuzumab producing CHO clone

Protein Characterization

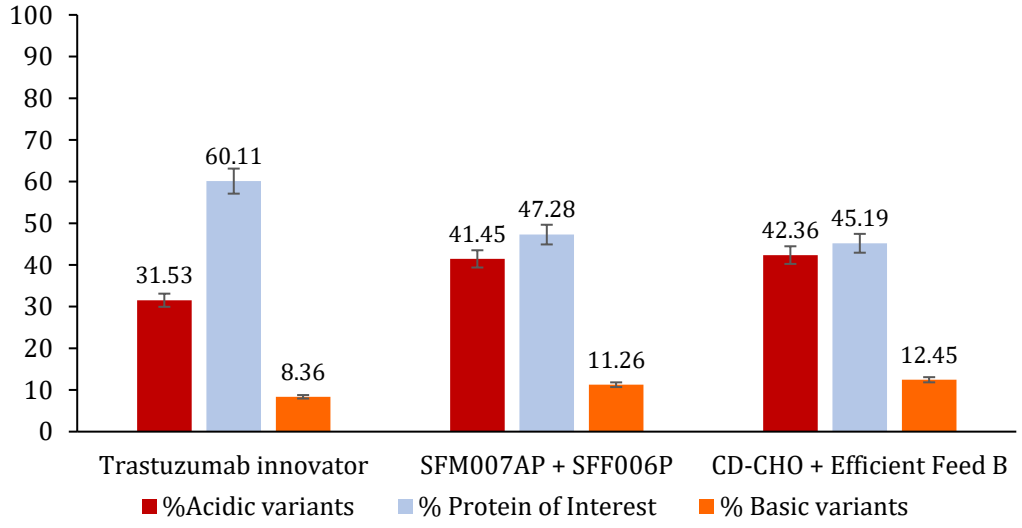
Aggregation Analysis

	Total Aggregates (%)	Protein of interest %	% Fragmentation
Trastuzumab innovator	1.002	98.5	0.498
SFM007AP + SFF006P	1.316	98.08	0.604
CD-CHO + Feed	1.306	98.444	0.250



Charge Variant Analysis

	%Acidic variants	% Protein of Interest	% Basic variants
Trastuzumab innovator	31.53	60.11	8.36
SFM007AP + SFF006P	41.45	47.28	11.26
CD-CHO + Feed	42.36	45.19	12.45



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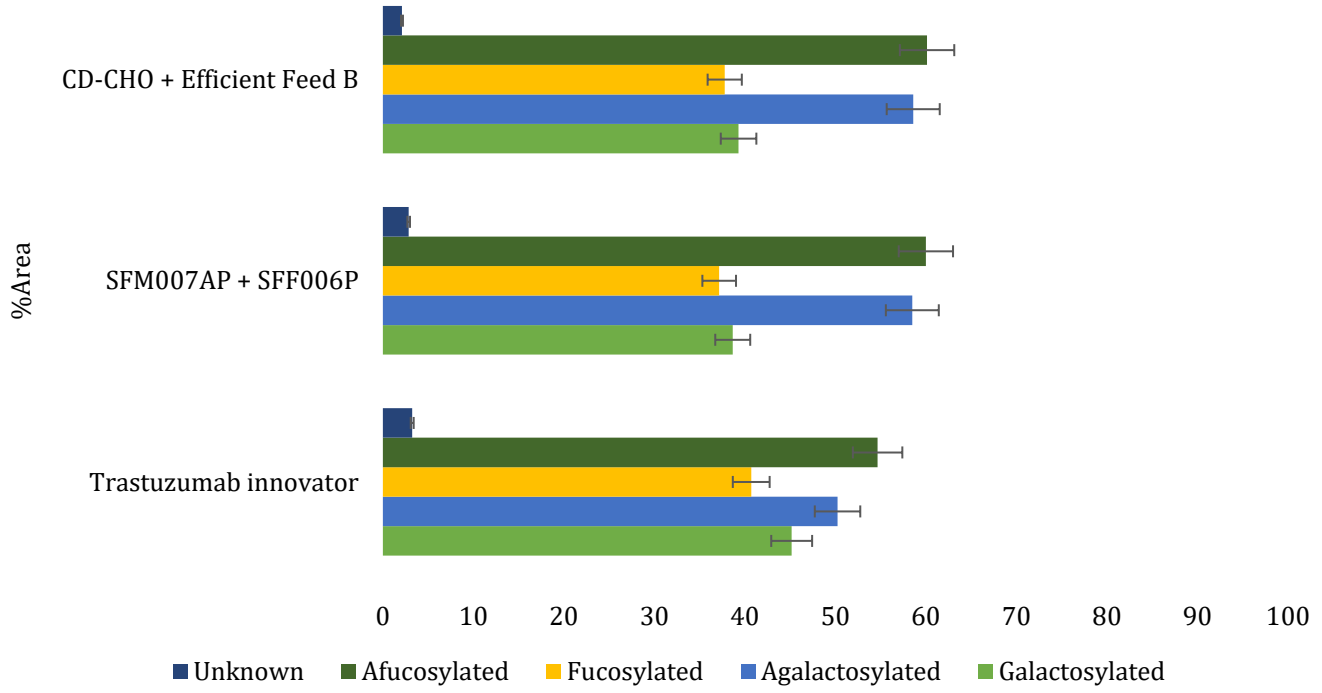
Trastuzumab producing CHO clone

Glycan Pattern

Glycan type	% Area		
	Trastuzumab innovator	SFM007AP + SFF006P	CD-CHO + Efficient Feed B
Galactosylated	45.165	38.653	39.297
Agalactosylated	50.231	58.495	58.592
Fucosylated	40.696	37.156	37.767
Afucosylated	54.657	59.992	60.122
Unknown	3.24	2.852	2.112



- Data represented over here is of 10L Bioreactor scale level

Potency and functional analysis

Test	Acceptance criteria #	HiMedia SFM007L + SFF001P	Competitor Basal medium + Feed supplement
Purity by SDS PAGE	-	Purity more than 80%	Purity more than 80%
Identity by Western Blot	-	Distinct 50kDa and 25kDa bands observed	Distinct 50kDa and 25kDa bands observed
Concentration by BCA assay	-	1.18mg/ml	1.08mg/ml
Residual CHO HCP	-	< 100ppm	< 100ppm
Residual CHO HCDNA	-	< 100ng/ dose	< 100ng/ dose
Isotyping (IgG subclass detection)	-	IgG1	IgG1
Biological potency by ADCC assay *	0.8 – 1.2	0.952	0.805
Binding potential by Her2 ECD Biomarker assay *	80 – 120%	94.9%	92.5%

References

1. European Pharmacopoeia Chapter 5.3, Statistical Analysis of Results of Biological Assays and Tests.
2. United States Pharmacopeia Chapters Bioassays <111> ,<1030>, <1032>, <1033>, <1034>
3. Assay Acceptance Criteria for Multiwell-Plate–Based Biological Potency Assays -Draft for Consultation. BioProcess International 12(1) January 2014 pages 30-41 www.bioprocessintl.com/journal/2014/January/Assay-Acceptance-Criteria-for-Multiwell-PlateBased-Biological-Potency-Assays-349245.
4. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology (http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf).
5. https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM273910.pdf
6. USP <1033> Validation of Biological Assays

BEVACIZUMAB

CLONE

PERFORMANCE CHARACTERISTICS

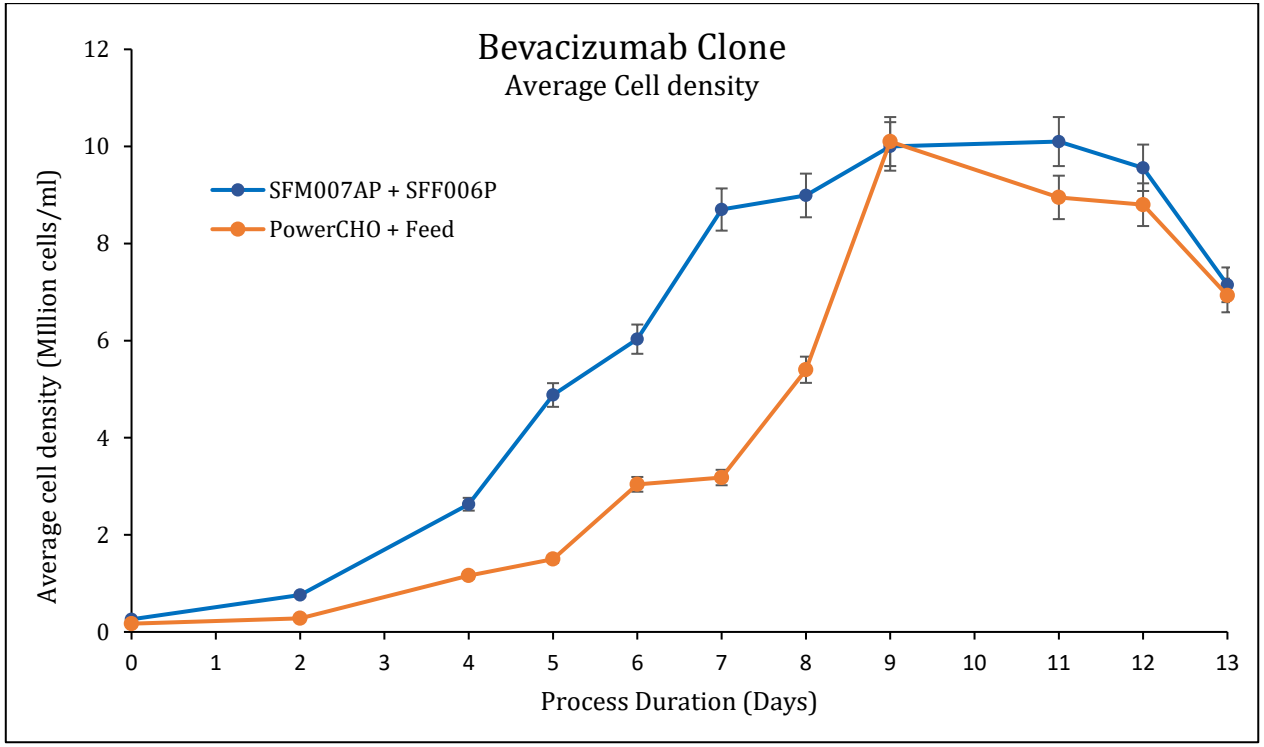
Bevacizumab producing CHO clone

Experimental conditions

Culture parameters	Details
Culture Vessel	: Scale up from Bioreactor tubes (50ml) to Sartorius B-Twin **10L Bioreactors** Data represented here is for Sartorius B-Twin **10L Bioreactors**
Process type	: Fed-batch culture
Combination details	1. Medium SFM007AP + Feed (SFF006P) 2. PowerCHO (Lonza) + Feed
Cell line	: Recombinant CHO clone producing mAb Bevacizumab
Culture conditions	: 80rpm, 37°C
Instruments used	1. Sartorius B-Twin 10L Bioreactors 2. Biochemical analyzer (Nova Biomedical)
No. of vessels per combination	: 1
Seeding density	: 0.5 million cells per ml
Seeding viability	: 93.14%
Duration of experiment	: 12 - 15 days
Test parameters and frequency	1. Cell density: Daily 2. Cell viability: Daily 3. IVCC: Daily 4. IgG Titer: Alternate day after Day 8 5. IgG Characterization: Harvested sample 6. IgG potency testing and functional assays

Cell density (Million cells/ml)

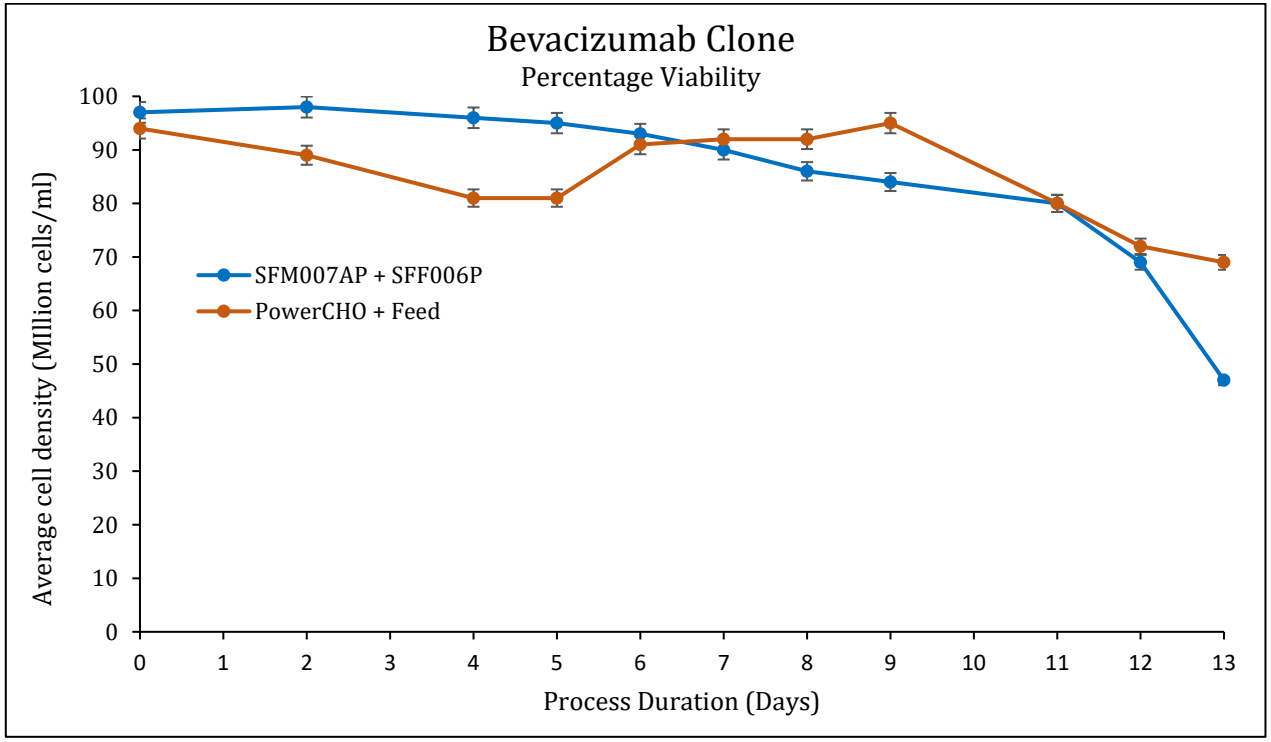
Combination	Incubation time (Day)										
	0	2	4	5	6	7	8	9	11	12	13
SFM007AP + SFF006P	0.46	0.76	2.63	4.88	6.03	8.70	7.68	10.00	6.10	5.20	5.10
PowerCHO + Feed	0.47	0.28	1.16	1.50	3.04	3.18	5.40	10.10	8.95	8.80	6.93



Peak density: 10 to 11 million cells/ml

Cell viability (Percentage)

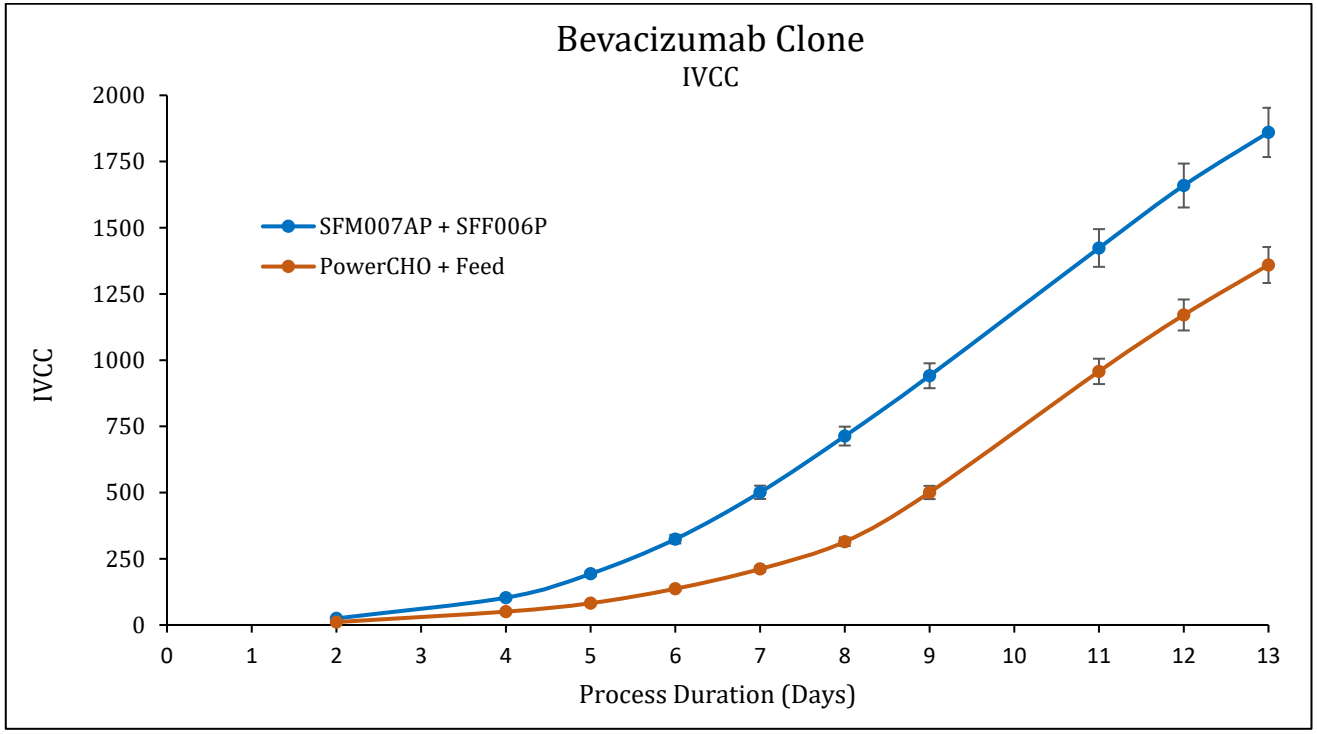
Combination	Incubation time (Day)										
	0	2	4	5	6	7	8	9	11	12	13
SFM033L + SFF001P	97	98	96	95	93	90	86	84	80	69	47
PowerCHO+ Feed	94	89	81	81	91	92	92	95	80	72	69



Sustained Cell Viability till Day 12

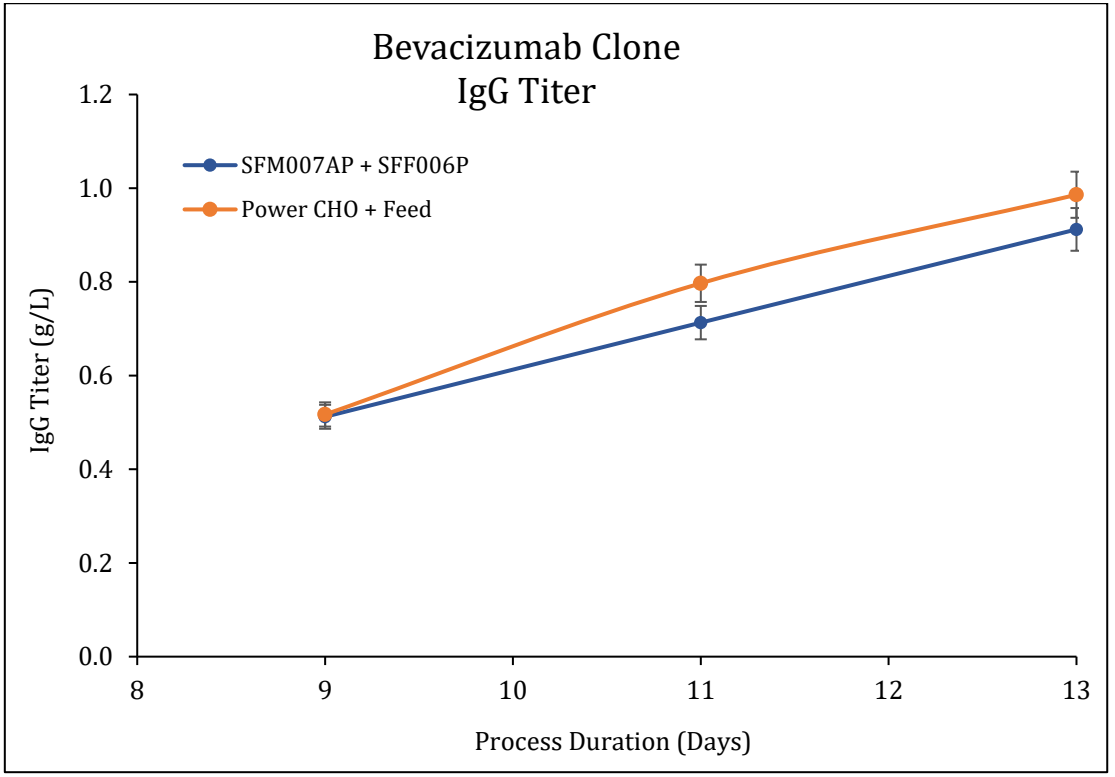
IVCC

Combination	Incubation time (Day)									
	2	4	5	6	7	8	9	11	12	13
SFM033L + SFF001P	24.74	103.16	193.28	324.20	500.96	713.24	941.12	1423.52	1659.44	1859.96
PowerCHO+ Feed	10.97	50.36	82.28	136.76	211.40	314.36	500.36	957.56	1170.56	1359.32



IgG Titer

Combination	Incubation time (Day)		
	9	11	13
SFM032L + SFF001P	0.512	0.713	0.912
PowerCHO + Feed	0.517	0.797	0.986



PERFORMANCE SUMMARY

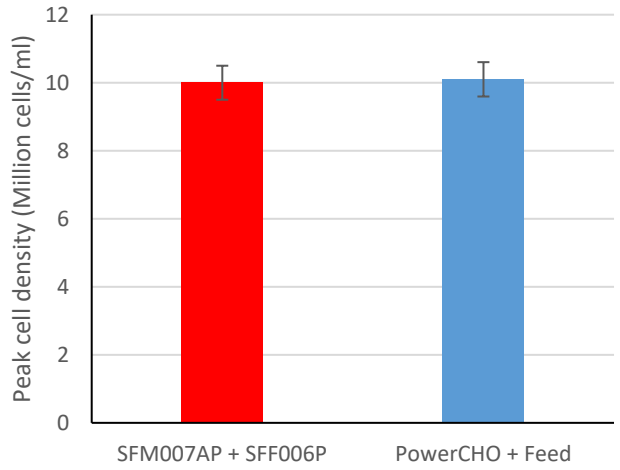
Bevacizumab producing CHO clone

Cultural Characteristics

Test parameter	HiMedia SFM007AP + SFF006P	Thermo PowerCHO + Feed
Peak cell density	10 – 11 million cells / ml	10 – 11 million cells / ml
Peak cell viability	More than 80%	More than 80%
IgG titer	0.912g/L	0.986g/L
Specific productivity	13.1 pg/ cell / day	13.3 pg/ cell / day

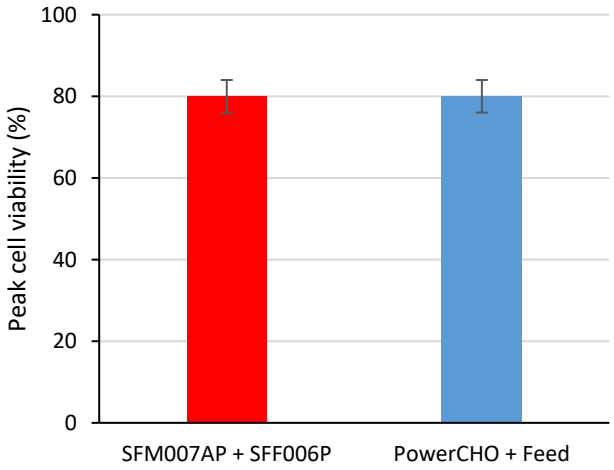
Peak Cell Density

10 – 11 million cells/ml on Day 10



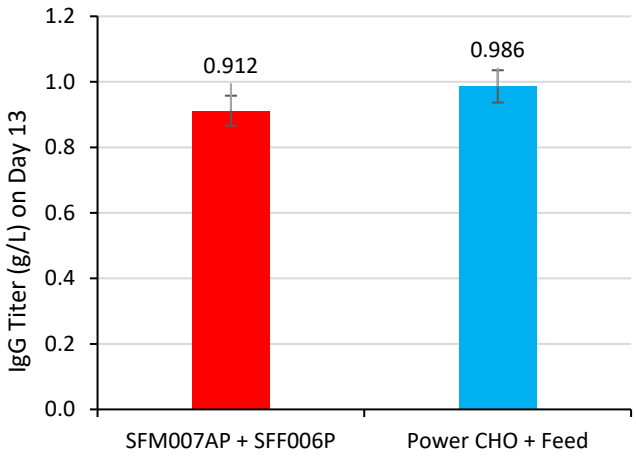
Cell Viability

More than 80% viability up to Day 11



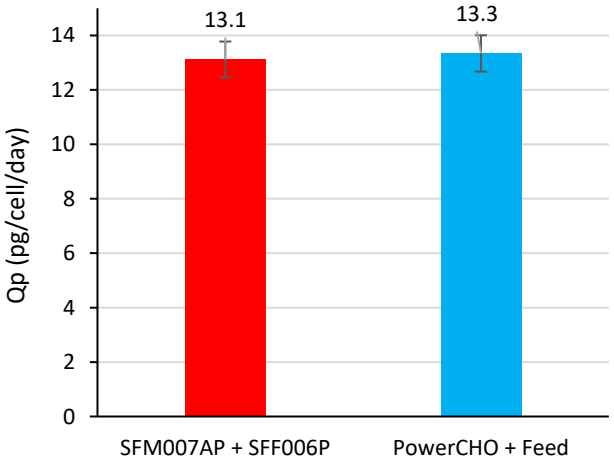
IgG Titer

~ 1g/L IgG Titer on Day 13



Specific Productivity

Qp > 13 pg per cell per day

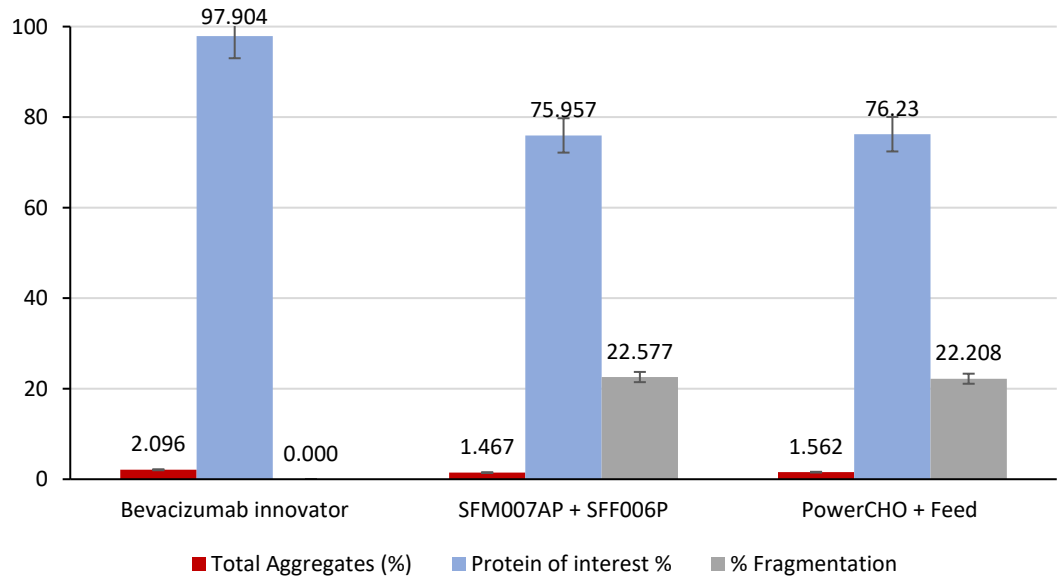


❖ Data represented over here is of 10L Bioreactor scale level

Protein Characterization

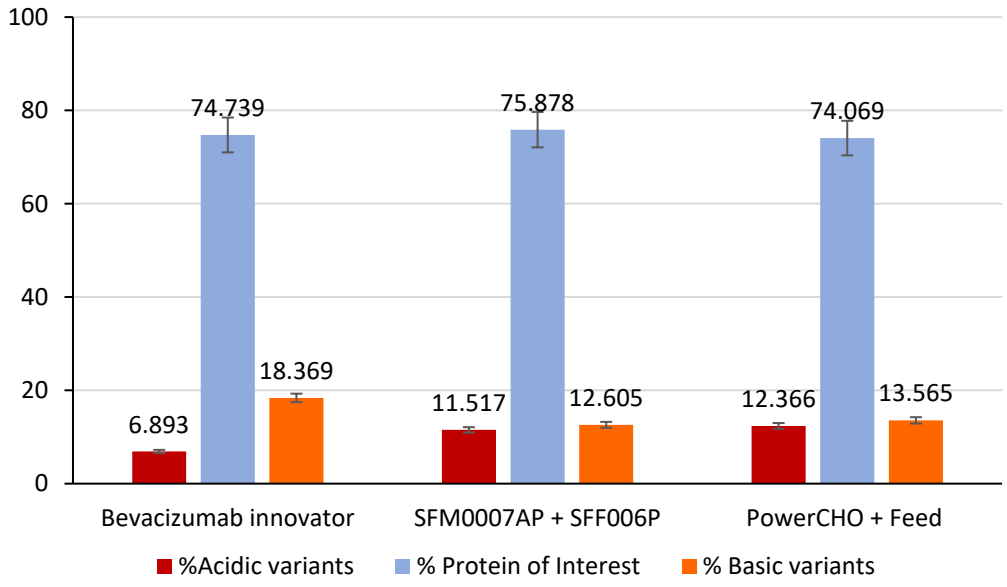
Aggregation Analysis

	Total Aggregates (%)	Protein of interest %	% Fragmentation
Bevacizumab innovator	2.096	97.904	0.000
SFM007AP + SFF006P	1.467	75.957	22.577
PowerCHO + Feed	1.562	76.23	22.208



Charge Variant Analysis

	%Acidic variants	% Protein of Interest	% Basic variants
Bevacizumab innovator	6.893	74.739	18.369
SFM007AP + SFF006P	11.517	75.878	12.605
PowerCHO + Feed	12.366	74.069	13.565

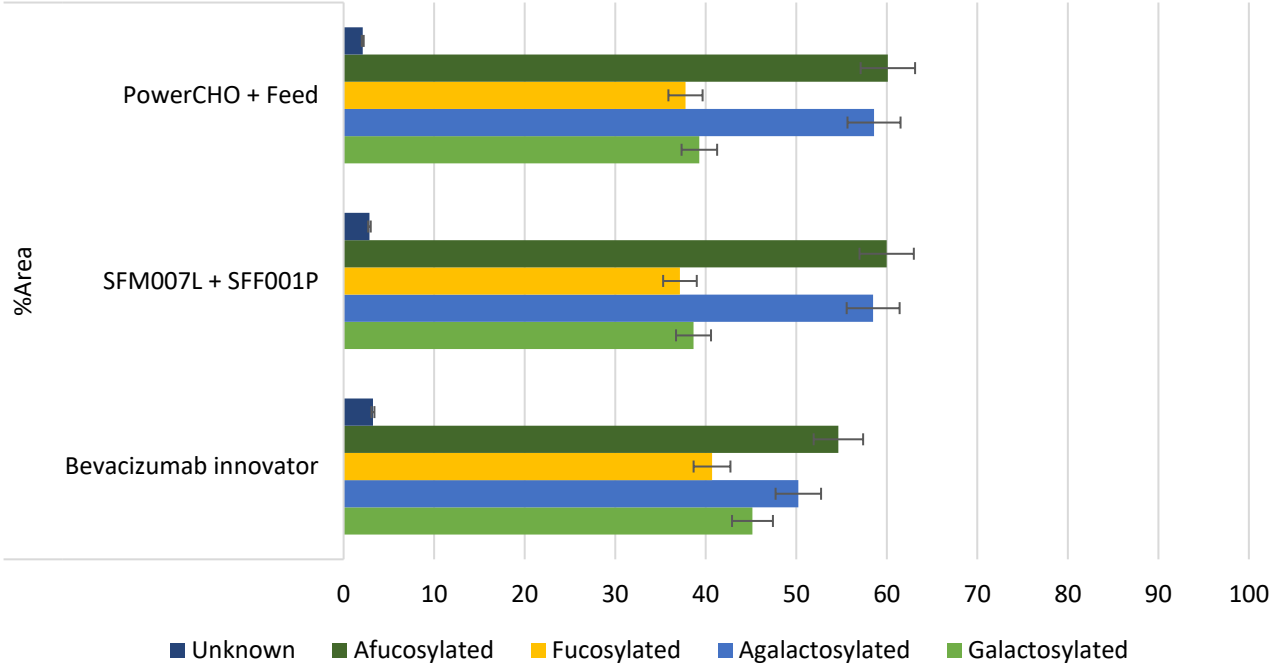


- Data represented over here is of 10L Bioreactor scale level

Bevacizumab producing CHO clone

Glycan Pattern

Glycan type	%Area		
	Bevacizumab innovator	SFM007AP + SFF006P	PowerCHO + Feed
Galactosylated	45.165	38.653	39.297
Agalactosylated	50.231	58.495	58.592
Fucosylated	40.696	37.156	37.767
Afucosylated	54.657	59.992	60.122
Unknown	3.24	2.852	2.112



❖ Data represented over here is of 10L Bioreactor scale level

Bevacizumab producing CHO clone

Potency and functional analysis

Test	HiMedia SFM007AP + SFF006P	PowerCHO + Feed supplement
Purity by SDS PAGE	Purity more than 80%	Purity more than 80%
Identity by Western Blot	Distinct 50kDa and 25kDa bands observed	Distinct 50kDa and 25kDa bands observed
Concentration by BCA assay	1.11mg/ml	1.07mg/ml
Residual CHO HCP	< 100ppm	< 100ppm
Residual CHO HCDNA	< 100ng/ dose	< 100ng/ dose
Isotyping (IgG subclass detection)	IgG1	IgG1
Biological potency by ADCC assay	0.993	0.961
Binding potential by Her2 ECD Biomarker assay	87%#	85%#

CUSTOMIZED BIOPROCESS OPTIMIZATION & MEDIA DEVELOPMENT

SERVICES

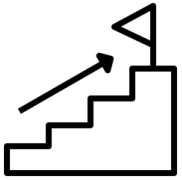
CUSTOM MEDIA and FEED MANUFACTURING SERVICE

Rely on us for optimizing media and feed for your high-value clones

3 MAJOR SERVICES PROVIDED BY HIMEDIA

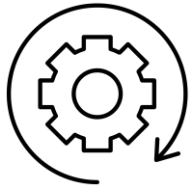
1) MEDIA OPTIMIZATION

We optimize media & feed to enhance the yield of therapeutic protein.



2) SCALE UP

We can optimize your process from R & D scale to pilot scale to mini production scale (50L). This can be further scaled up to large scale.



3) OPTIMIZE PROCESS PARAMETER

Our manufacturing capacity of customized media in single lot ranges from 3 kg to 5 tons.

CUSTOM MEDIA and FEED MANUFACTURING SERVICE

CLONE SECURITY

At the core of a bio manufacturing process for recombinant proteins is the production cell line. It influences the productivity and product quality. Its characteristics also dictate process development, as the process is optimized to complement the producing cell to achieve the target productivity and quality.

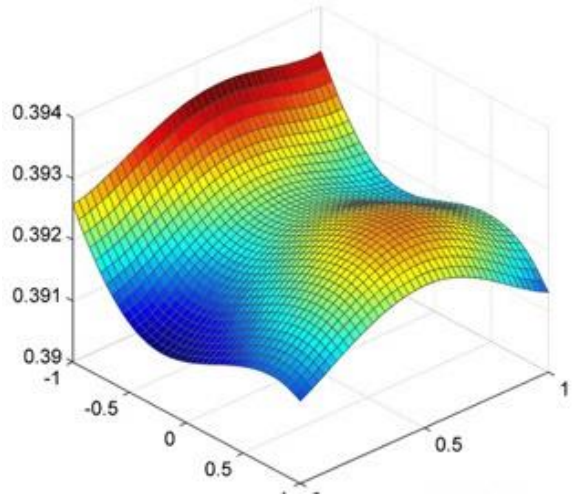
Understanding the complexity and criticality of clone development procedure, we are firmly committed to honor your intellectual property. We have a complete understanding of the confidentiality of your process. All your information is handled confidentially and we assure you that it will never be disclosed. We sign a confidentiality agreement to ensure that the integrity of your clone and process is not compromised.

Each recombinant CHO clone engineered to produce therapeutic proteins exhibits unique metabolic demands. Optimal productivity of each clone at large scale requires clone-specific medium and feed.

With the help “Design of Experiments”, our scientists follow a systematic approach for customization of media and feed. In a comprehensive approach, our R&D scientists can create an optimal nutritional environment, developing media formulations specifically suited to your working clone. We can formulate nutrient supplements to your specifications for bioreactor feed strategies.

Accuracy of the results is further assured by using High-throughput automated instruments and minimal manual intervention.

CUSTOM MANUFACTURING



SPENT MEDIA ANALYSIS SERVICE

Biochemical analysis

Nova BioProfile Flex 2

Estimation of primary indicators of culture kinetics

Glucose, Lactate, Glutamine, Ammonia, Sodium, potassium, Calcium, pO_2, pCO_2

Amino acid analysis

Sykam Amino Acid Analyzer

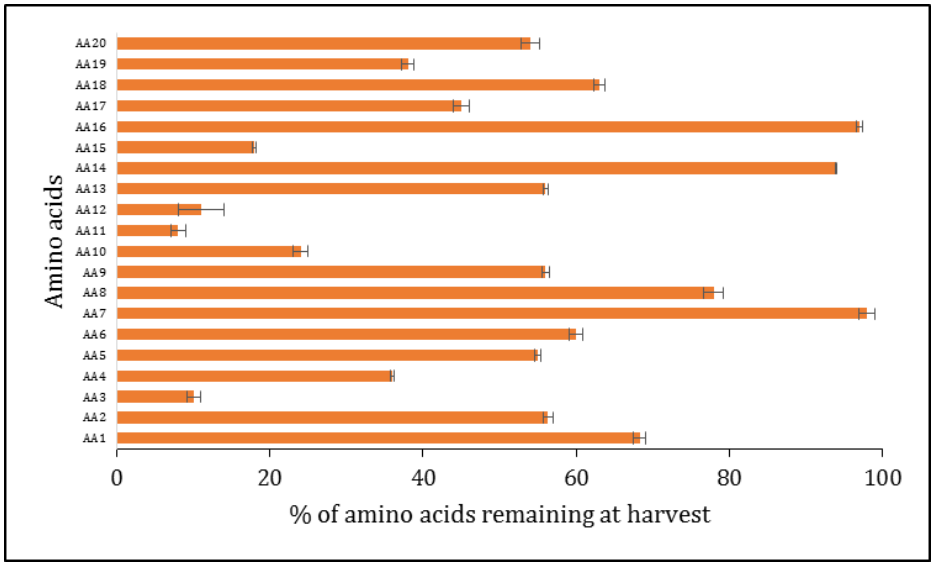
Estimation of rate-limiting and productivity influencing factors

Free amino acids

SPENT MEDIA ANALYSIS SERVICE

Amino acid analysis *Sykam Amino Acid Analyzer*

Aspartic acid	Valine
Threonine	Methionine
Serine	Isoleucine
Glutamic acid	Leucine
Glutamine	Tyrosine
Proline	Phenylalanine
Glycine	Histidine
Alanine	Tryptophan
Cysteine	Lysine
Ammonia	Arginine



Limit of detection
~100nM

HIMEDIA®
For Life is Precious

CELLBIO™

With immense pleasure we announce the

BIRAC INNOVATOR AWARD - 2020

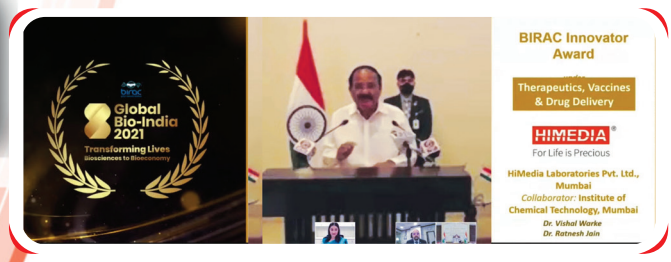
Awarded To

HiMedia Laboratories Pvt. Ltd.

For

We congratulate the entire team who are involved in this Innovation project

The award was presented by Shri M. Venkaiah Naidu, Hon'ble Vice President of India and Dr. Harsh Vardhan Hon'ble Union Minister for Science & Technology, Earth Sciences and Health & Family Welfare, India, in presence of Mr. Uday Kotak, President CII, Dr. Kiran Mazumdar Shaw, Chairperson & Managing Director, Biocon Limited., India and Dr. Roderico H Ofrin, WHO Representative to India.



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in Thane

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