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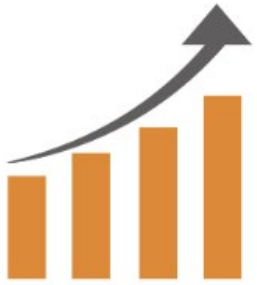
TunaCHO™ for Transient Protein & Antibody Production

NR5370.20190918v19

CHO Expression Systems at LakePharma



TunaCHO™

7-14 Day Transient
High Yield

10 mL – 20 L

Can reach >1 g/L

CHO-GSN™

High Performance
Stable

Can reach up to 7 g/L

Multiple programs
at clinical stage

- Support discovery, development, and manufacturing
- The same CHO-K1 parental is used for all platforms so products from the different platforms have similar activity & post-translational modification (PTM) profiles

Introducing TunaCHO – A High Yield Transient Production System

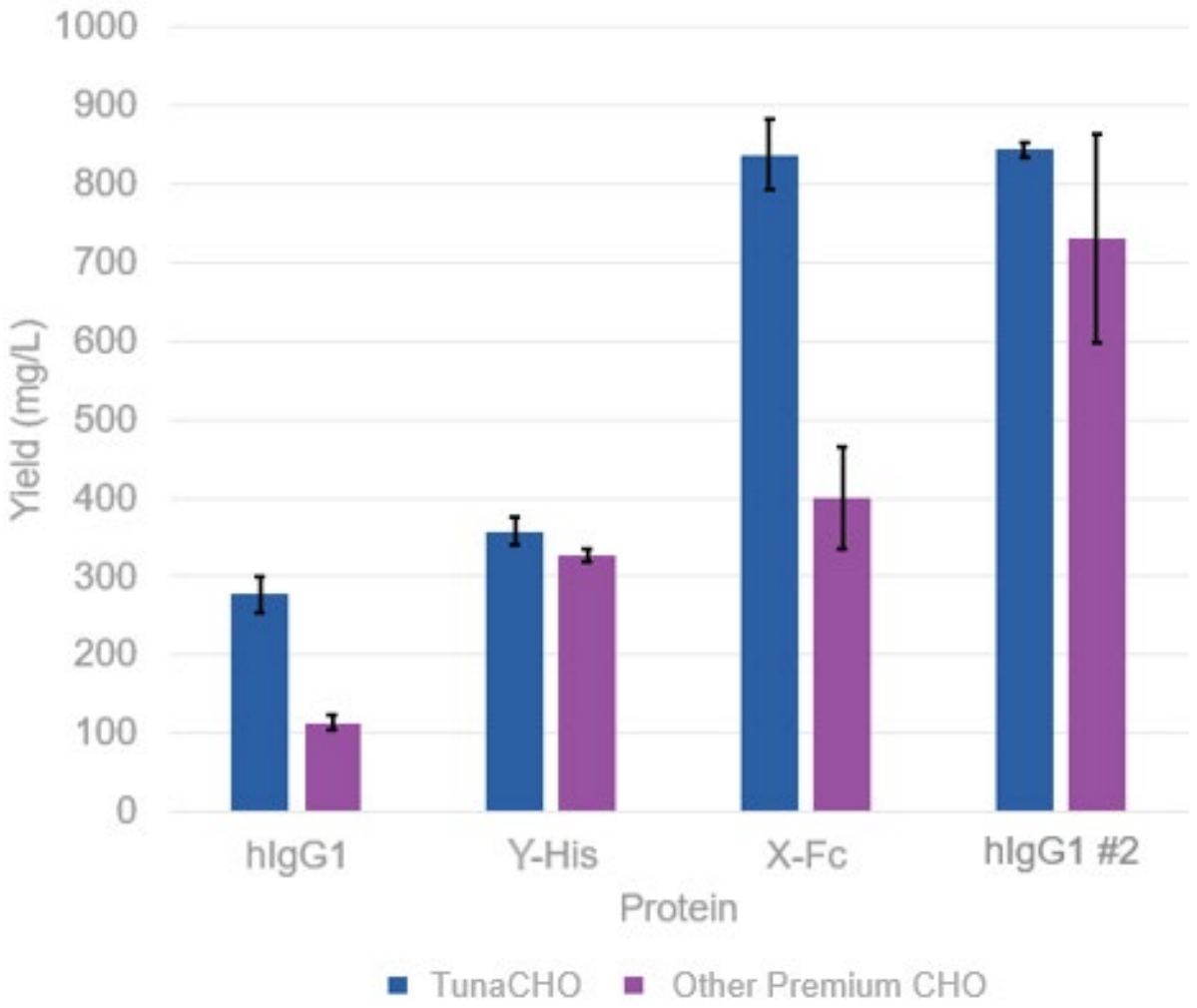
TunaCHO is a CHO transient protein production system

- LakePharma's proprietary CHO-SE™ cell line (same parental cell line as CHO-GSN™)
- MEDNA Bio reagents
- LakePharma's proprietary process

Compared to other CHO transient production systems, TunaCHO offers several distinctive advantages:

- Good productivity: can reach 2 g/L
- Consistent and scalable: same results from 10 mL to 20 L
- Cost effective compared to other premium CHO transient production systems

TunaCHO for High Productivity & Consistency



- TunaCHO as an alternative to other premium CHO transient systems in production of:
  - Recombinant human IgG1
  - Fc-fusion protein
  - His tagged non-antibody protein
- **TunaCHO process is consistent in production yield**

Data from extended 14-day production using TunaCHO is shown.

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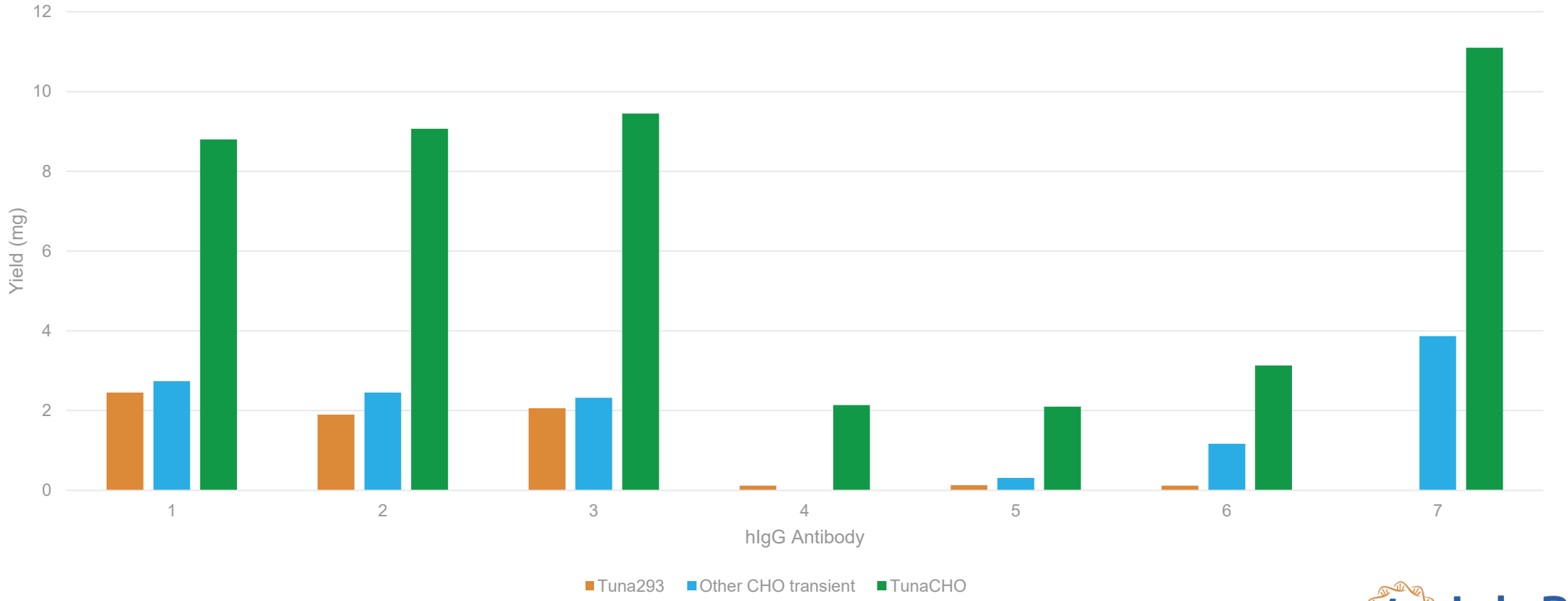
High Productivity with TunaCHO

Production of 7 different human IgG antibodies in 3 transient expression systems demonstrates TunaCHO has **high productivity**.

The TunaCHO platform is able to increase expression level of low-expressing antibodies (samples 4 – 6) by 10- to 20-fold. Each sample is from a 10 mL production volume.



Data from extended 14-day production using TunaCHO is shown.

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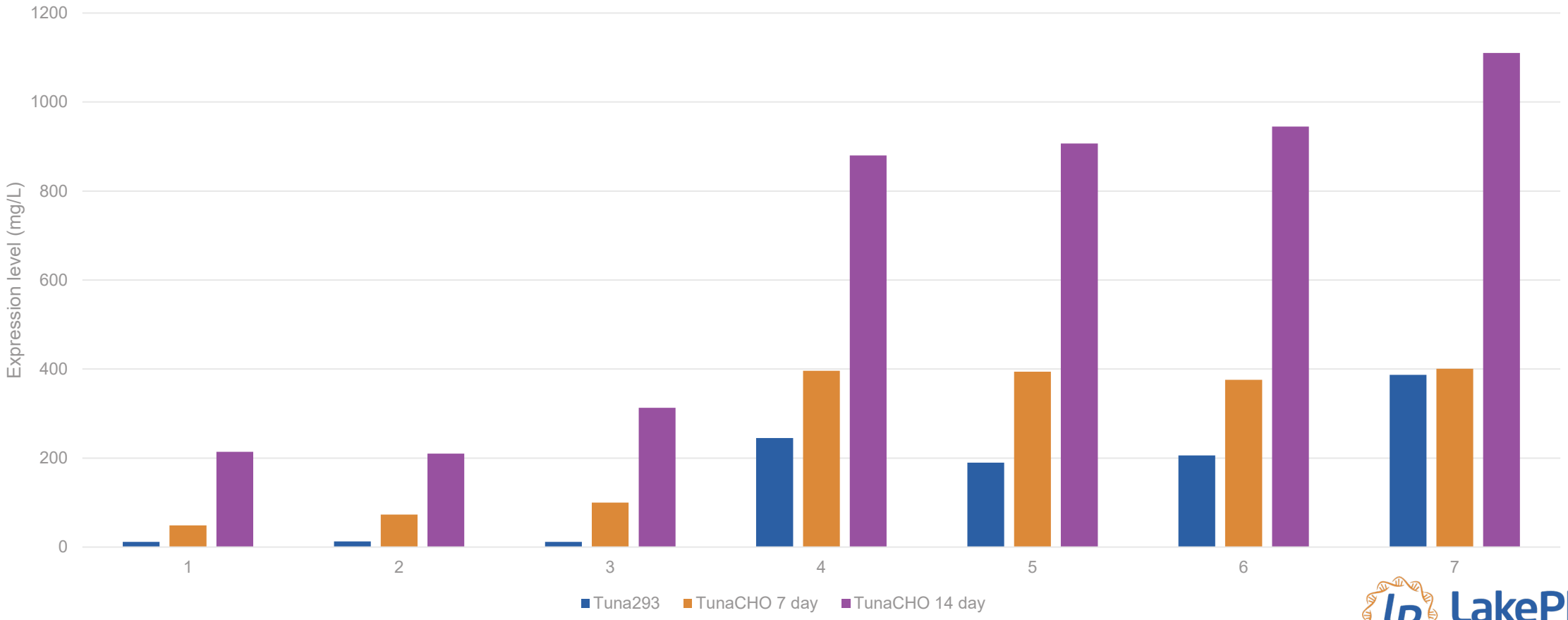
High Productivity with TunaCHO

Production of 7 different human IgG antibodies in Tuna293, TunaCHO (7 days), and TunaCHO (14 days extended run) transient expression systems is shown.

The TunaCHO 7-day platform is able to increase expression level of low-expressing antibodies (samples 1 – 3) by 10- to 20-fold while maintaining the expression level of a high-expressing antibody (sample 7).



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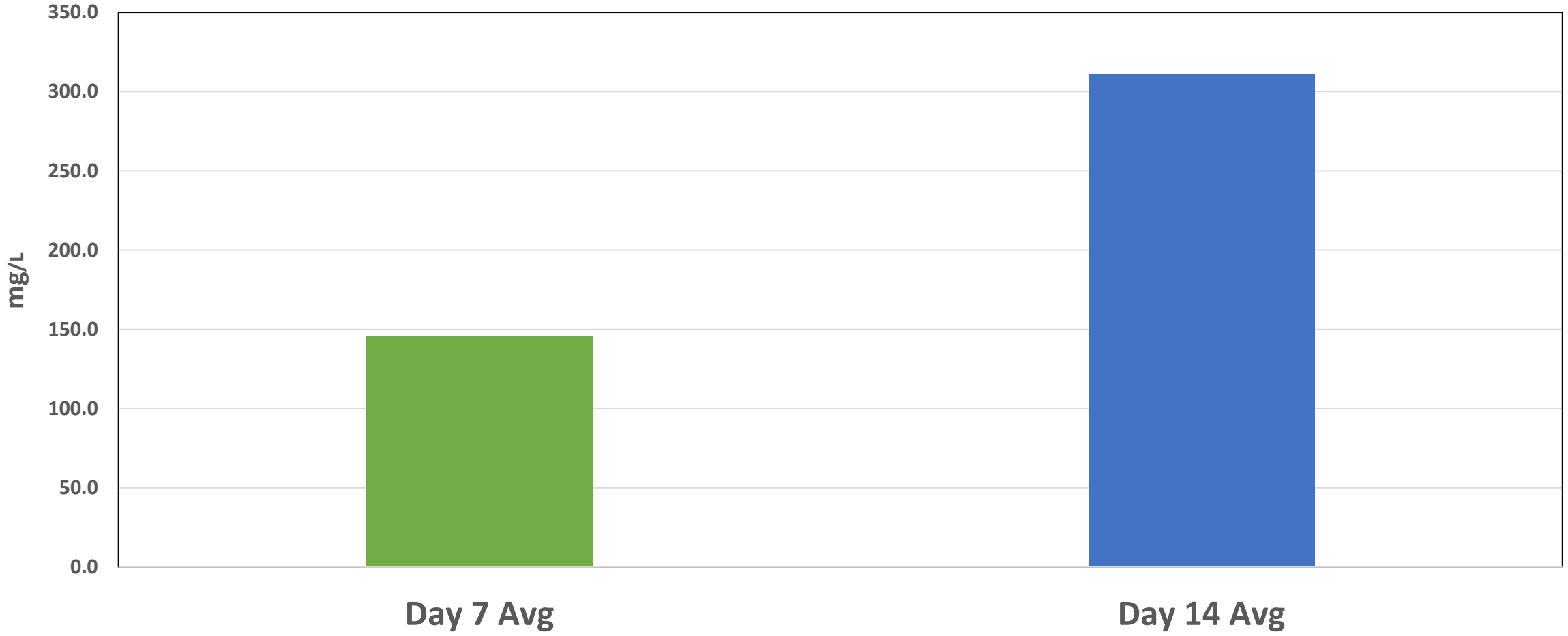
Average TunaCHO Yield 7 Days vs 14 Days*



* Data from >30 production runs including human antibodies, mouse antibodies, and Fc-fusion proteins. Non-Fc proteins were not included.

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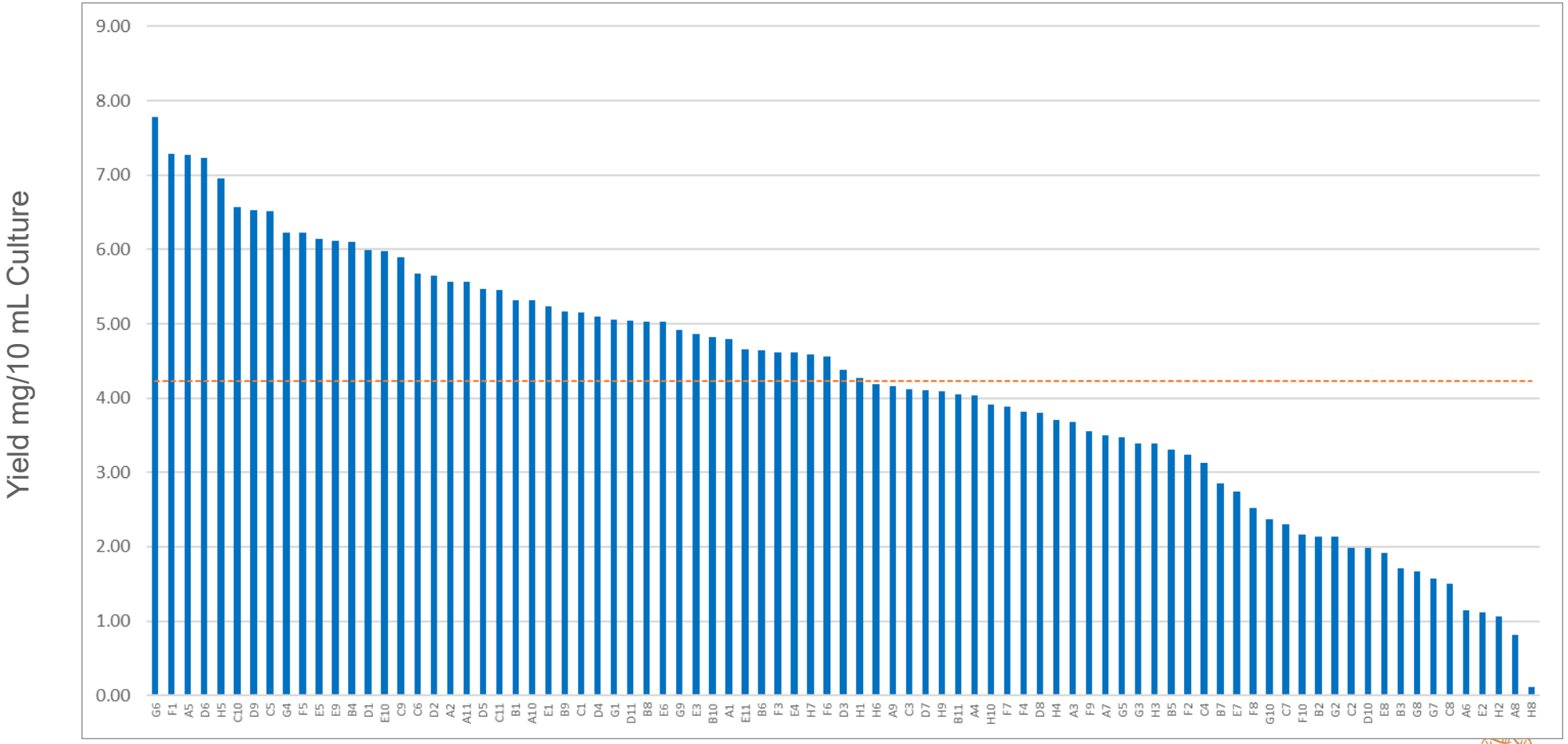
TunaCHO for High-Throughput Antibody Production

85 recombinant antibodies are produced in 10 mL TunaCHO process with Protein A purification.

Average yield is 4.2 mg per 10 mL culture.



Data from extended 14-day production using TunaCHO is shown.

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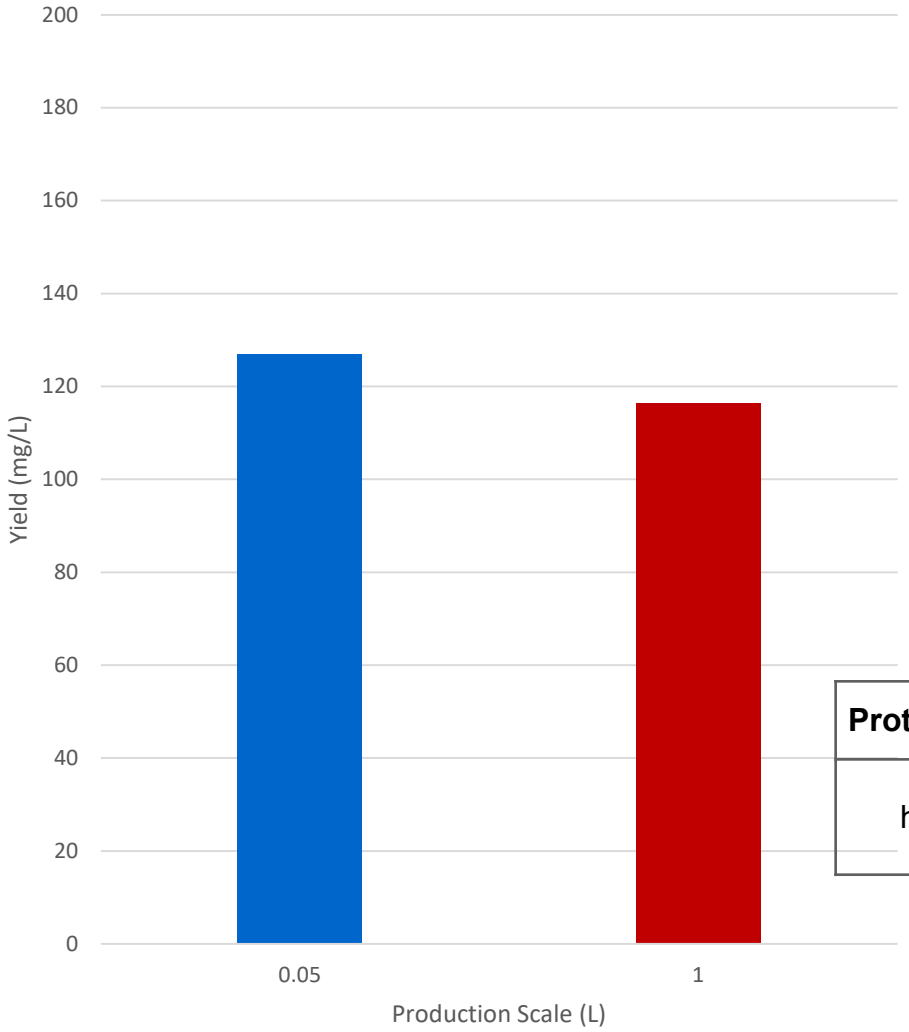
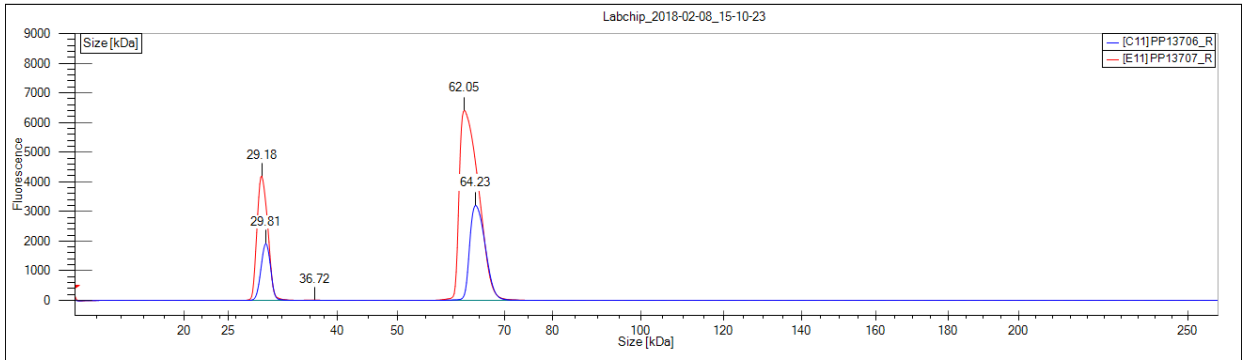
TunaCHO for Consistency and Scalability

Consistent TunaCHO production levels, CE-SDS profile, and intact mass results between 0.05 L and 1 L production of hIgG1 demonstrate consistency and scalability.





Protein Type	Production Volume	HC Measured	LC Measured	HC Calculated*	LC Calculated*	Δ HC	Δ LC
hIgG1	0.05L	48927	23947	48925	23947	2.0	0.3
	1L	48927	23947	48925	23947	2.0	0.3

Data from extended 14-day production using TunaCHO is shown.

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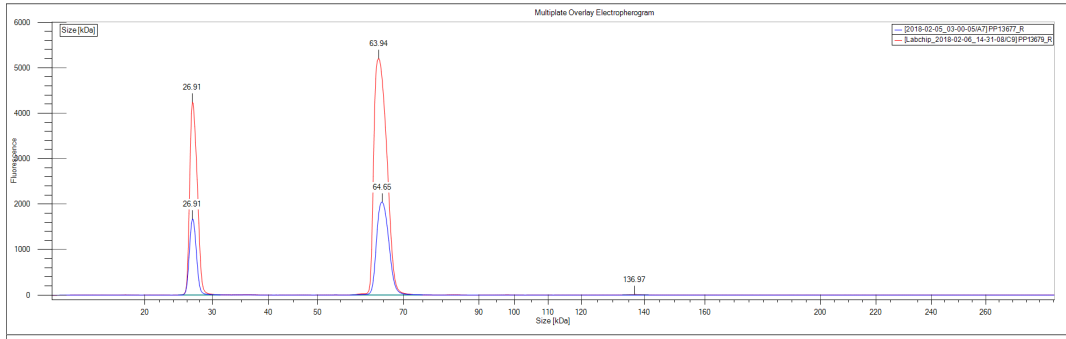
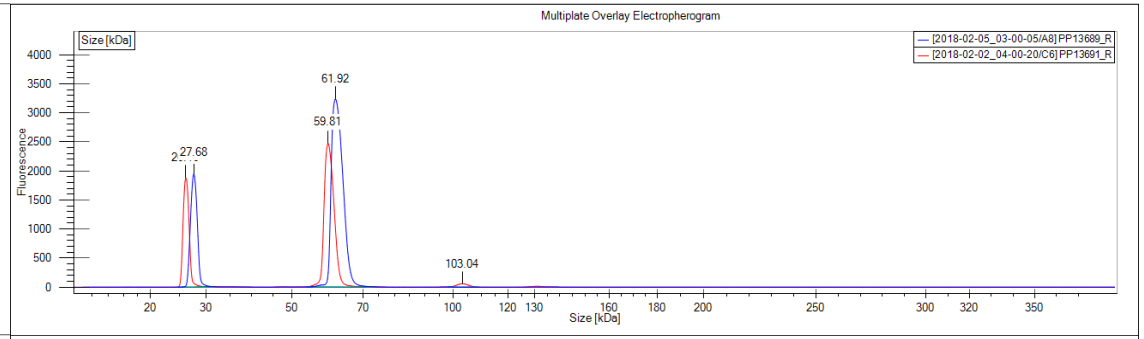
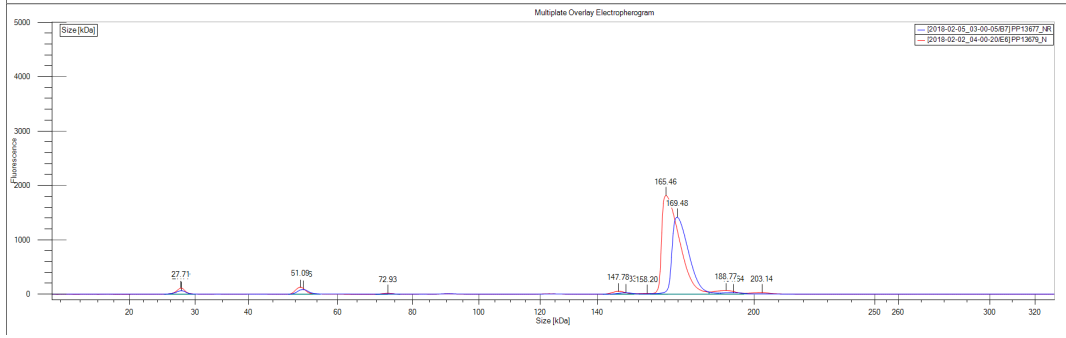
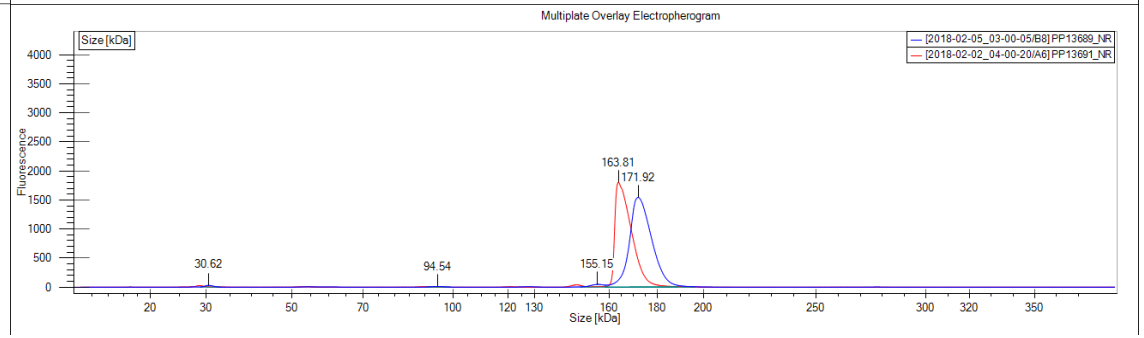
TunaCHO- Consistent CE-SDS Profile

CE-SDS was performed on antibodies produced from TunaCHO and another premium transient CHO system under reducing & non-reducing conditions.

	hIgG1 #1	hIgG1 #2
Reducing		
Non-Reducing		

TunaCHO

Other Premium Transient CHO System

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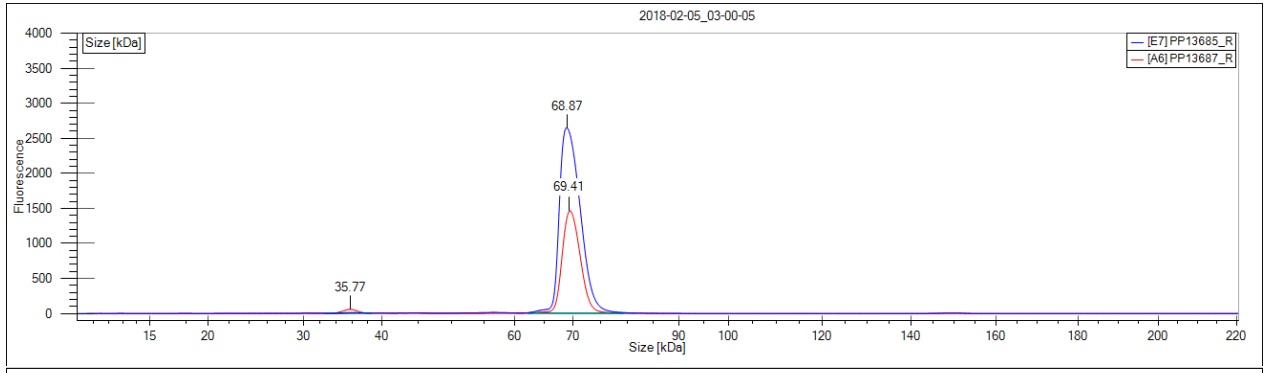
TunaCHO - Consistent CE-SDS Profile

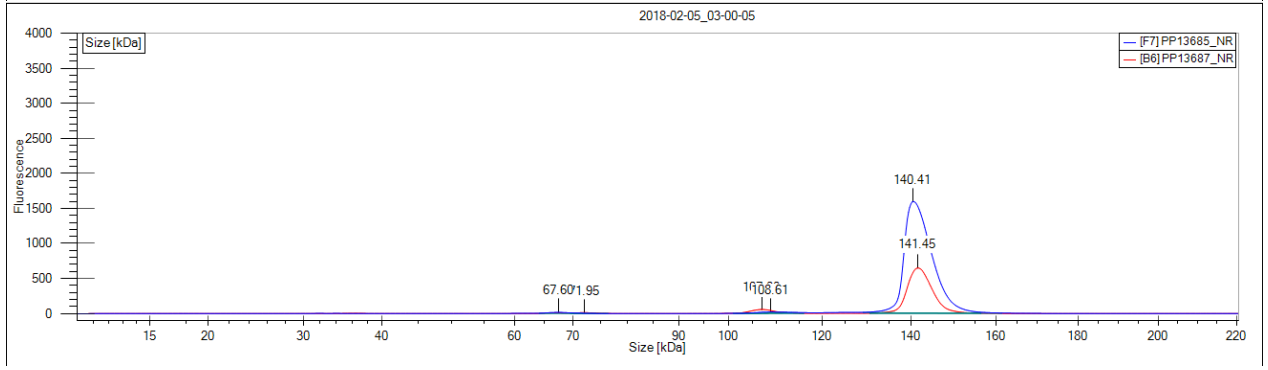
CE-SDS was performed for Fc-fusion proteins from TunaCHO and another premium transient CHO system under reducing & non-reducing conditions.

Fc-Fusion Protein

Reducing



Non-Reducing



TunaCHO

Other Premium Transient CHO System

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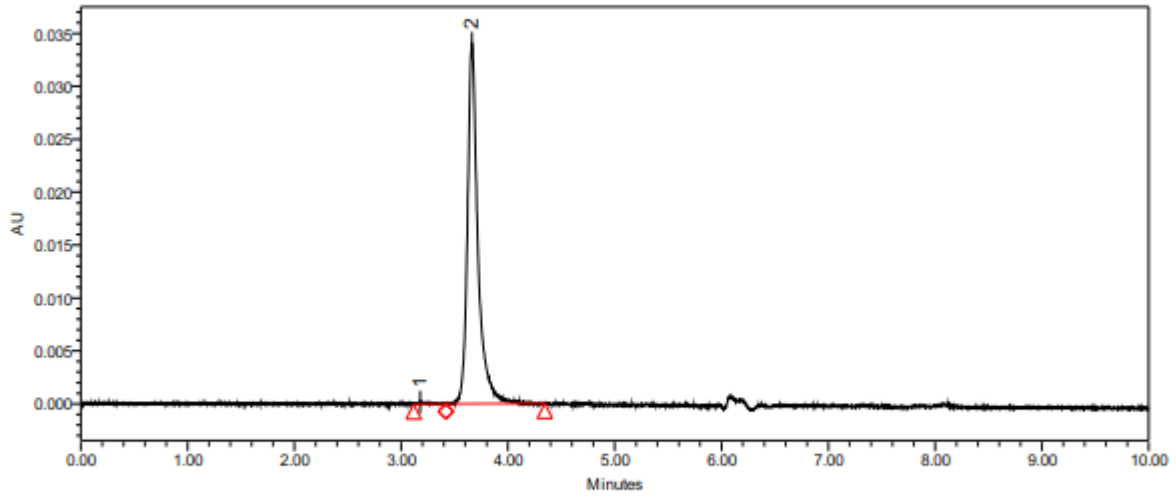
High Purity from TunaCHO Production

Two different antibodies were produced in TunaCHO at the 0.05 L production scale and purified via Protein A. SE-UPLC analysis demonstrates **production level does not impact purity.**

hIgG1 #1: >600 mg/L

Results

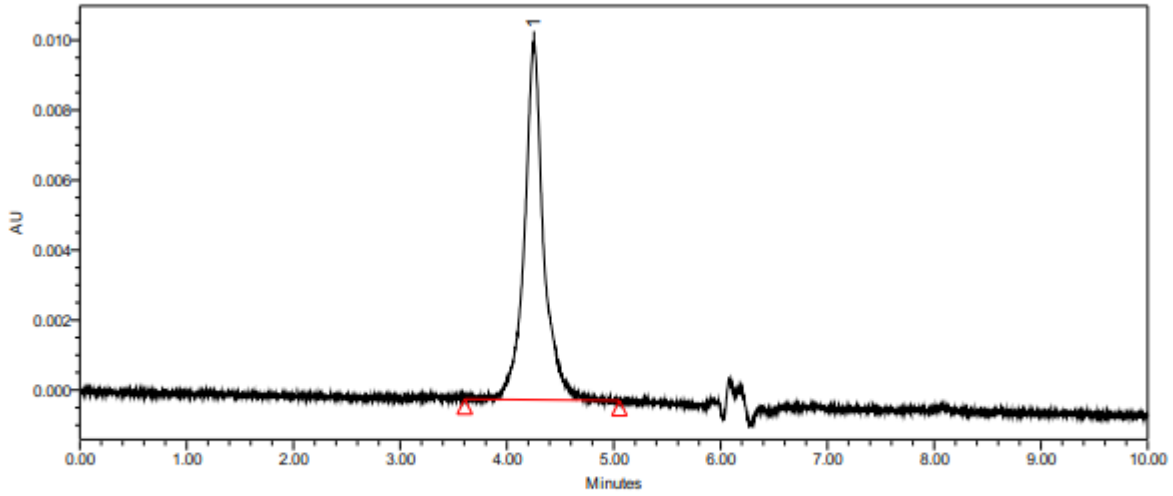


	Peak Label	RT (min)	% Area	Peak Size (kDa)	Name
1	1	3.180	0.38	---	Aggregate
2	2	3.662	99.62	---	Monomer

PP13505

hIgG1 #2: >260 mg/L

Results



	Peak Label	RT (min)	% Area	Peak Size (kDa)	Name
1	1	4.254	100.00	---	Monomer

PP13506

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TunaCHO - Consistent Intact Mass Results

TunaCHO productions (in duplicates) were compared to another premium transient CHO system to demonstrate **comparable intact mass profiles.**

Expression system	Protein Type & Label	HC Measured	LC Measured	HC Calculated*	LC Calculated*	Δ HC	Δ LC
Other premium transient CHO system	hIgG1 #1	49126	23444	49124	23443	2.0	1.2
		49126	23444	49124	23443	2.0	1.2
	hIgG1 #2	49280	23661	49279	23660	1.5	0.6
		49280	23661	49279	23660	1.5	0.6
	Fc fusion	51677	-	51676	-	1.3	0.0
		51677	-	51676	-	1.3	0.0
TunaCHO	hIgG1 #1	49126	23444	49124	23443	2.0	1.2
		49126	23444	49124	23443	2.0	1.2
	hIgG1 #2	49280	23661	49279	23660	1.5	0.6
		49280	23661	49279	23660	1.5	0.6
	Fc fusion	51678	-	51676	-	2.3	0.0
		51678	-	51676	-	2.3	0.0

All samples were run under deglycosylated & non-reduced conditions.

* The calculated M.W. includes mass shift of the pyroglutamate N-terminal Q and C-terminal lysine clipped based on the M.W. from DS.

Intact mass measurement of reduced and deglycosylated protein confirmed the sequence of each purified protein prep with delta M.W. less than 3 Da.

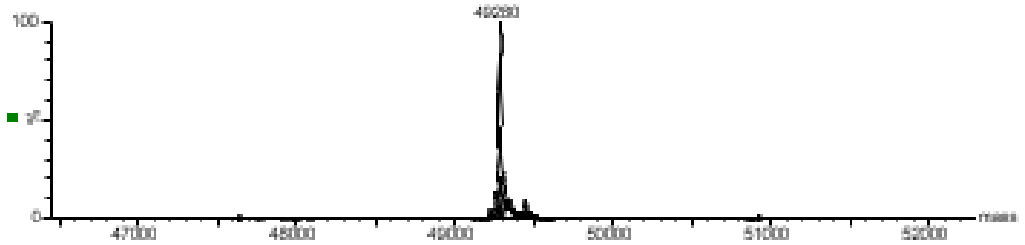
TunaCHO - Consistent Intact Mass Results

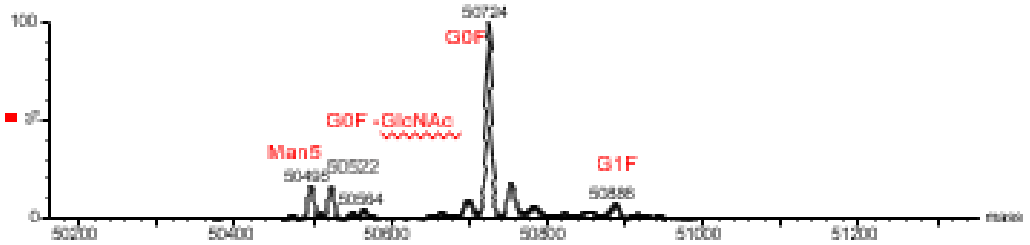
hIgG1

Reduced & Deglycosylated

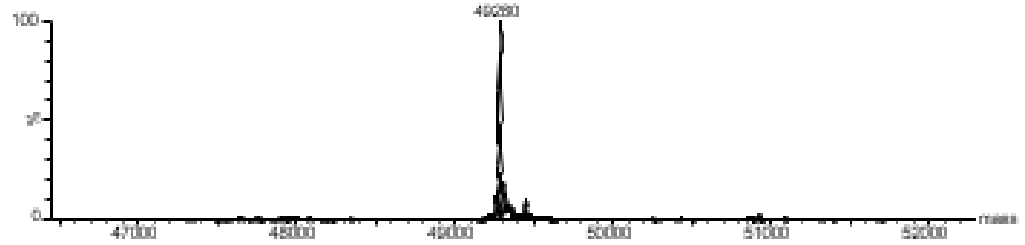
Reduced

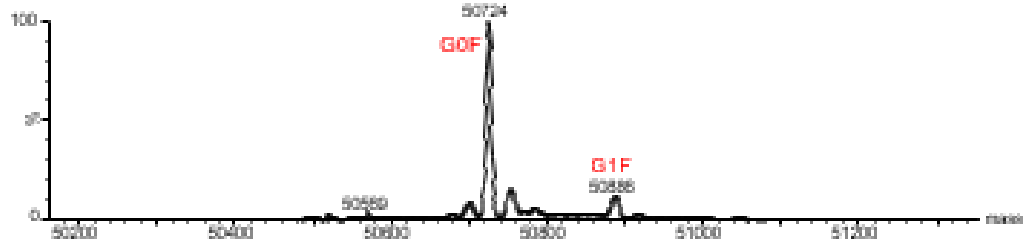
Other Premium CHO - HC



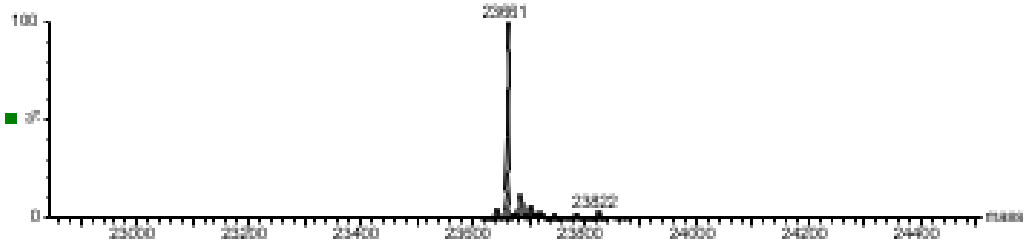


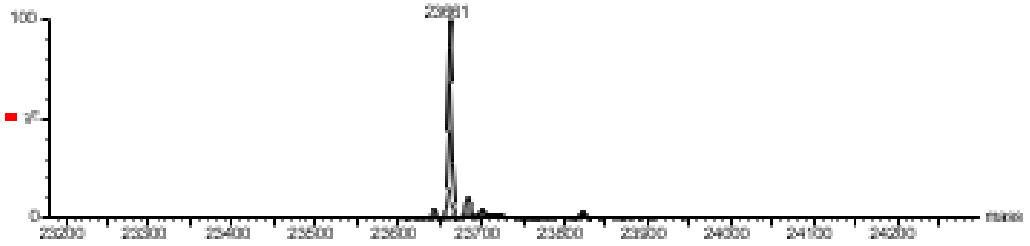
TunaCHO HC



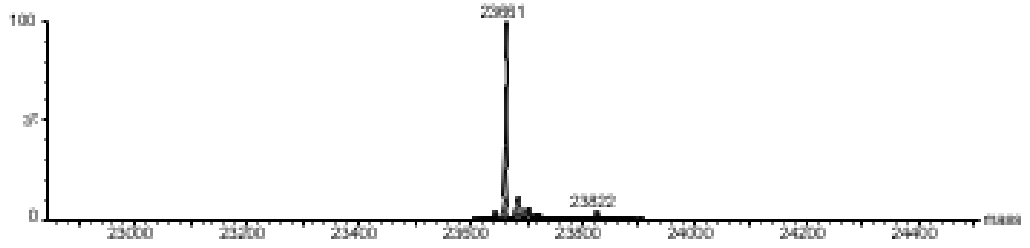


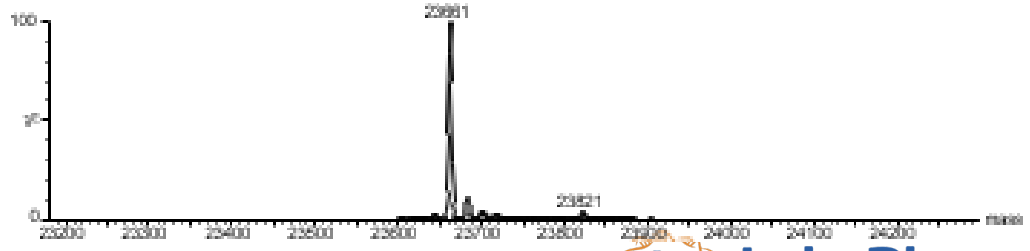
Other Premium CHO - LC





TunaCHO LC





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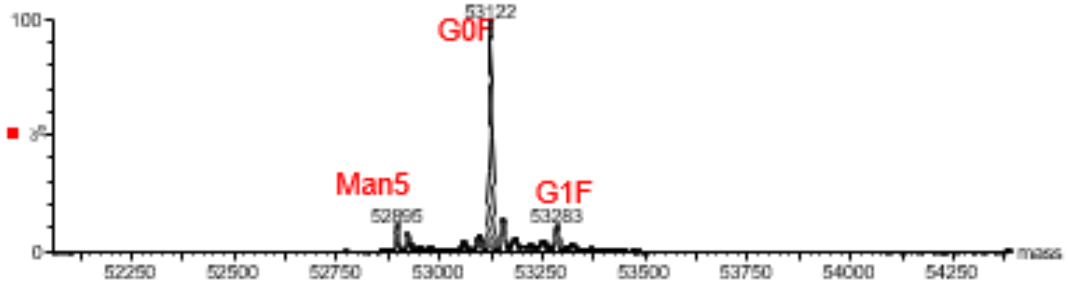
TunaCHO - Consistent Intact Mass Results

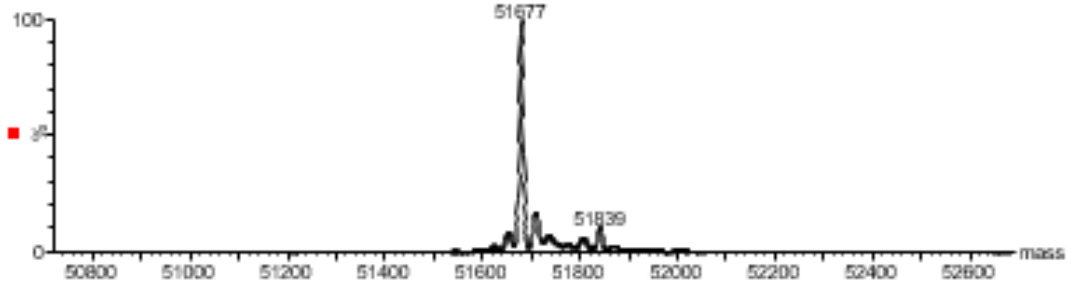
Fc-fusion Protein

Reduced

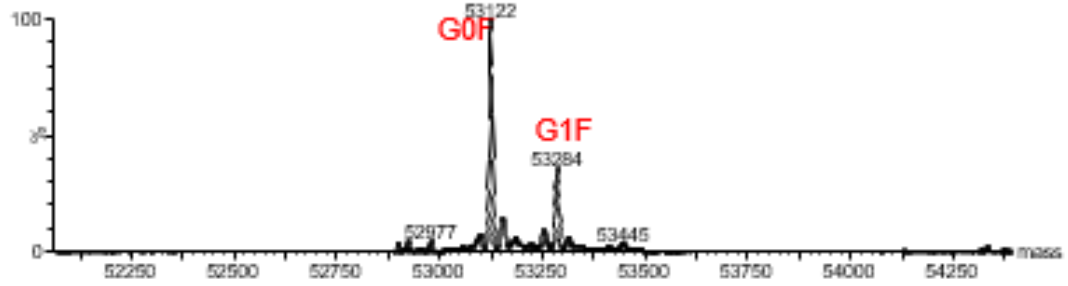
Reduced & Deglycosylated

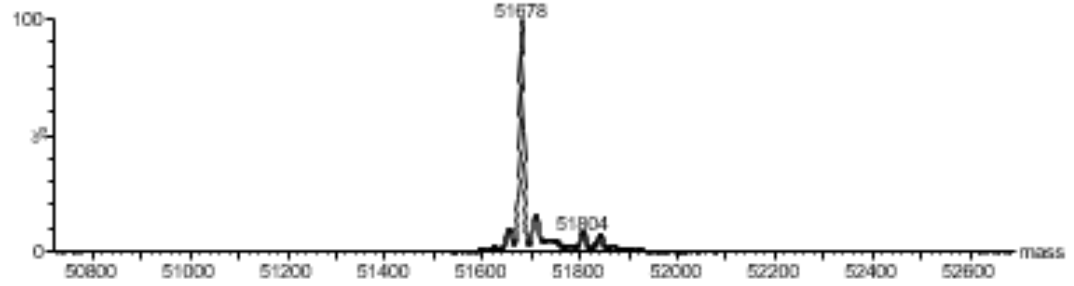
Other Premium CHO





TunaCHO





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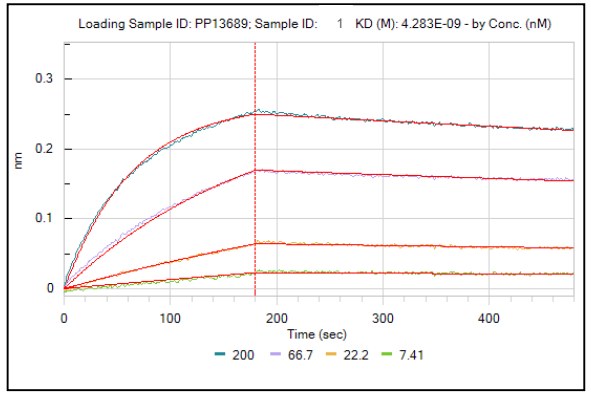
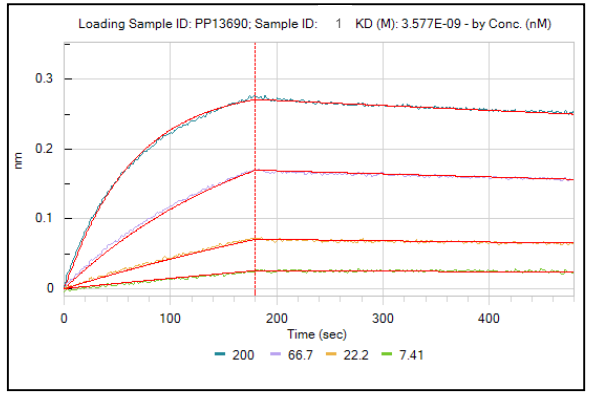
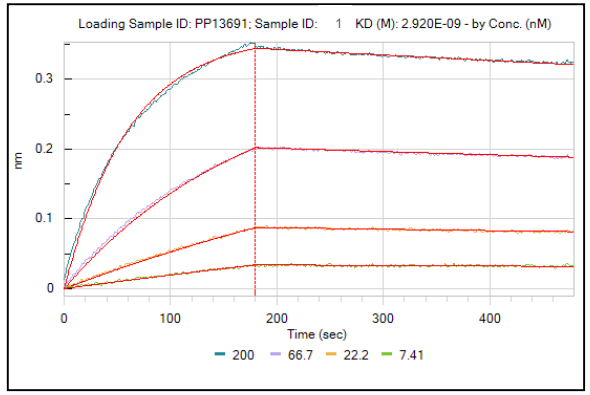
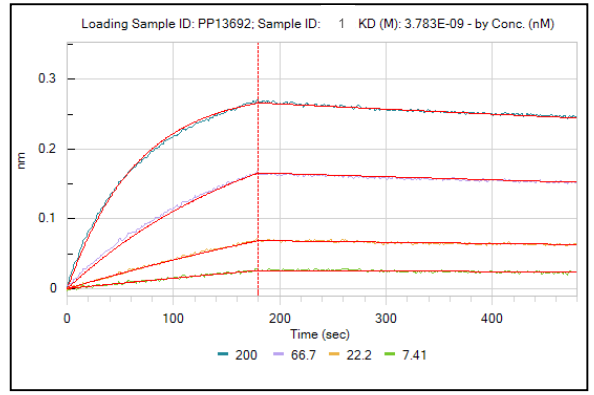
TunaCHO - Comparable Binding Affinity

TunaCHO productions (in duplicates) were compared to another premium transient CHO system to demonstrate **comparable affinity measurement results.** hIgG1 binds the antigen.









Loading Sample ID	TunaCHO		Other Premium CHO	
Sample ID	PP13689	PP13690	PP13691	PP13692
KD (M)	4.3E-09	3.6E-09	2.9E-09	3.8E-09
Kon (1/Ms)	7.6E+04	7.6E+04	8.1E+04	7.5E+04
Kdis (1/s)	3.3E-04	2.7E-04	2.4E-04	2.8E-04
Full X^2	0.0111	0.0096	0.0121	0.0086
Full R^2	0.9992	0.9994	0.9995	0.9994

Methods:
Binding experiments were performed on Octet® RED96 at 25°C. Purified antibodies (0.5 µg/mL) were loaded onto Anti-Human IgG Fc (AHC) biosensors. Loaded sensors were dipped into a three-fold dilution series of antigen "1" (starting at 200 nM). Kinetic constants were calculated using a monovalent (1:1) binding model.

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Available TunaCHO Transient Production Services

7-Day Standard Production

- Ideal for productions that need quicker turnaround time
- TunaCHO offers higher yields than HEK293

14-Day Extended Production

- Ideal for obtaining higher yields of proteins (2X higher than 7-day standard production)
- Over 2 to 10-fold higher than HEK293

For Antibody using TunaCHO

- Gene synthesis and pilot production
  Learn More >
- Large-scale production
  Learn More >

Non-antibody Proteins using TunaCHO

- Gene synthesis and pilot production
  Learn More >
- Large-scale production
  Learn More >

Working with LakePharma

- Comprehensive technology platform
- Technical consultation with experts in protein production
- Online data system for 24-hour access to project information (timelines, data, team communications)
- Strong project management with regular project updates (email and teleconferences)

For more information, please contact Inquiries@LakePharma.com