



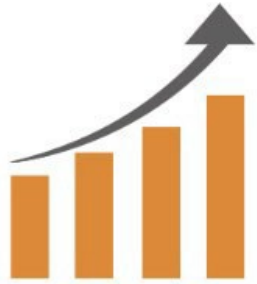
**LakePharma**  
*The Biologics Company*

# TunaCHO™ for Transient Protein & Antibody Production

NR5370.2020113v20



# CHO Expression Systems at LakePharma



## TunaCHO™

**7-14 Day Transient  
High Yield**

10 mL – 20L

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

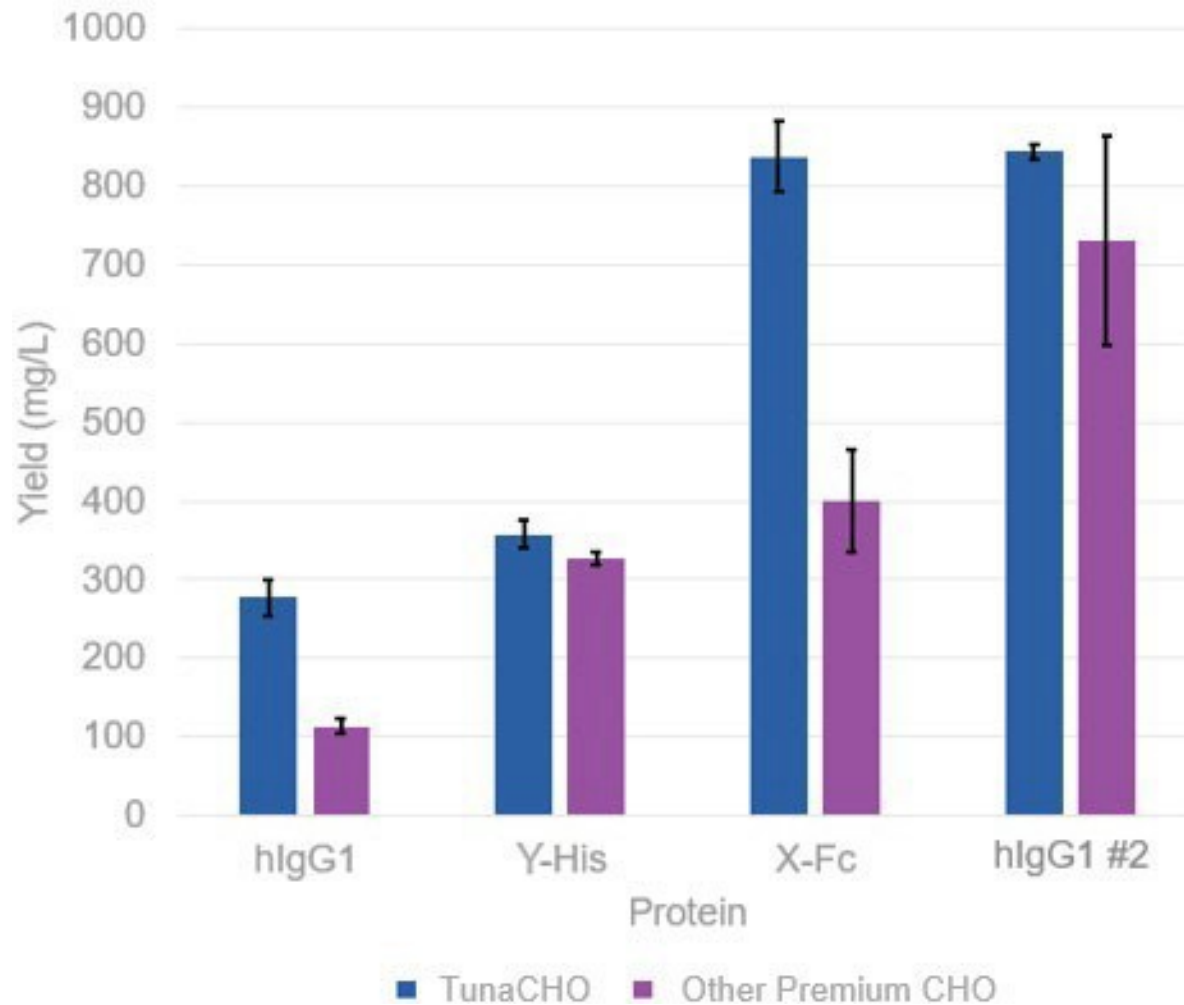
TunaCHO is a CHO transient protein production system

- 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 >1 g/L
- Consistent and scalable: same results from 10 mL to 20L
- Cost effective compared to other premium CHO transient production systems

# TunaCHO for High Productivity & Consistency



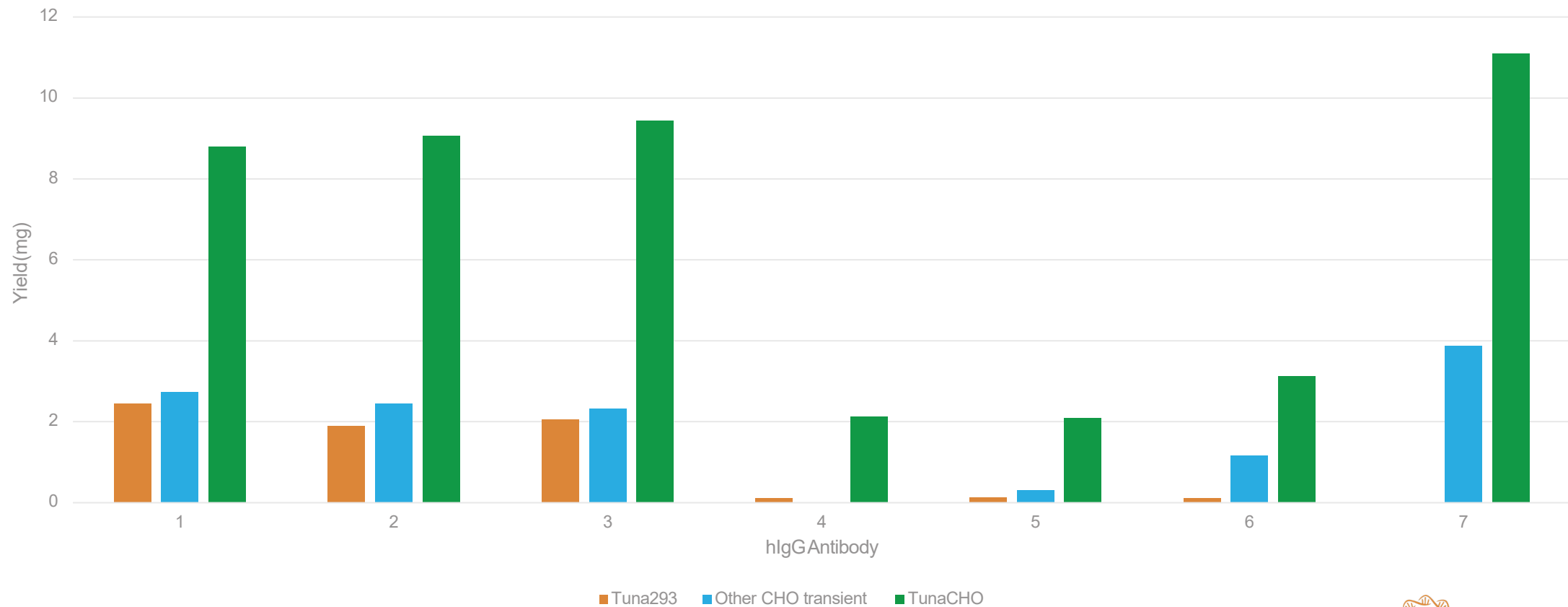
- TunaCHO as an alternative to other premium CHO transient systems in production of:
  - Recombinant human IgG1
  - Fc-fusion protein
  - His tagged non-antibody protein
- **TunaCHO process is consistent in production yield**

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

# 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.

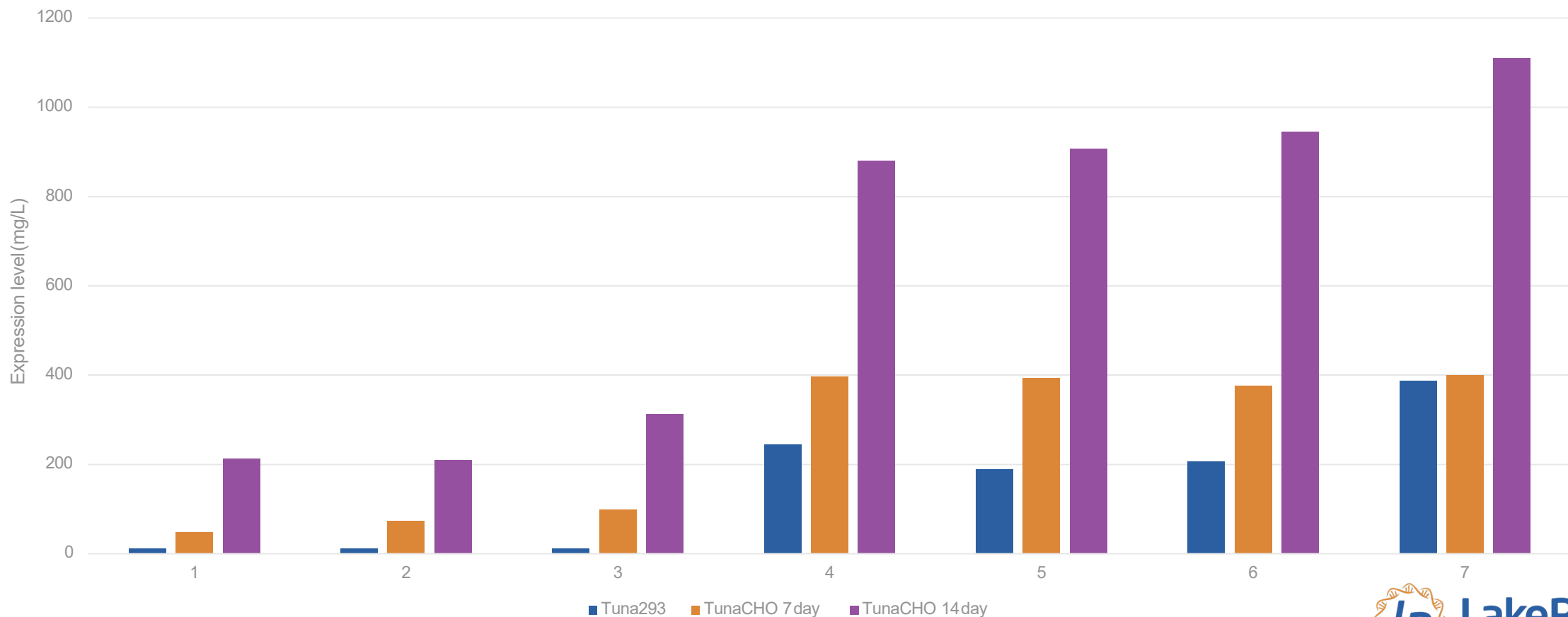


Data from extended 14-day production using TunaCHO is shown.

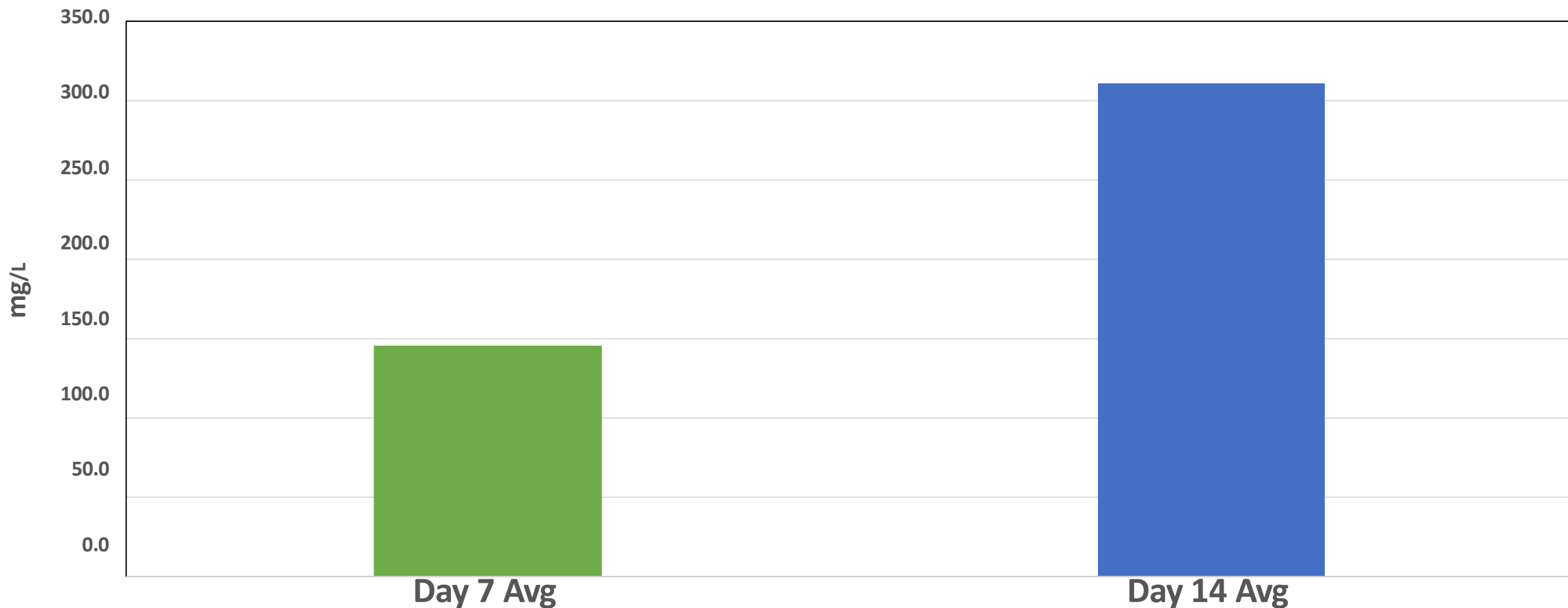
# High Productivity with TunaCHO

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).



## Average TunaCHO Yield 7 Days vs 14 Days\*

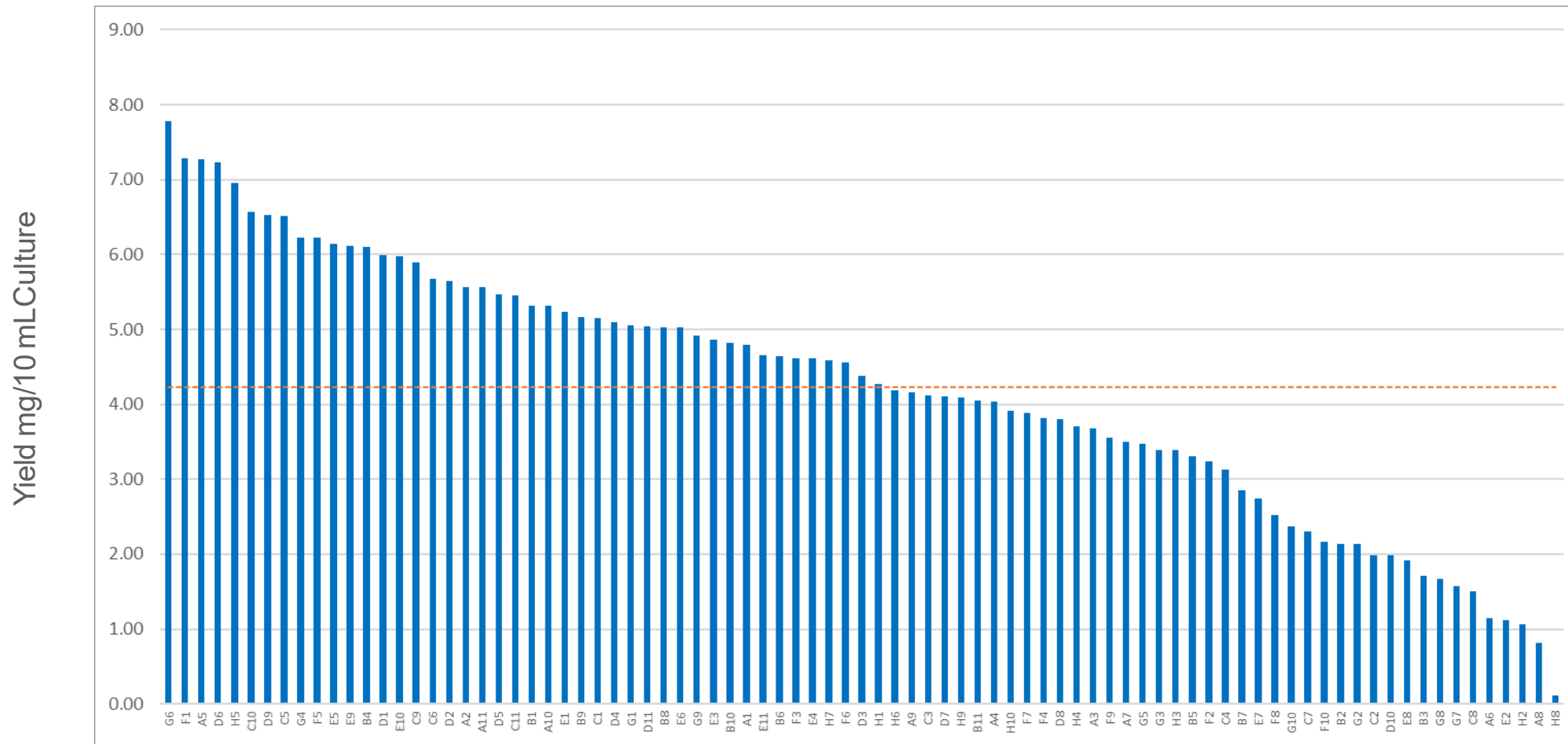


\* Data from >30 production runs including human antibodies, mouse antibodies, and Fc-fusion proteins. Non-Fc proteins were not included.

# TunaCHO for High-Throughput Antibody Production

85 recombinant antibodies are produced in 10 mL TunaCHO process with ProteinApurification.

Average yield is 4.2 mg per 10 mL culture.

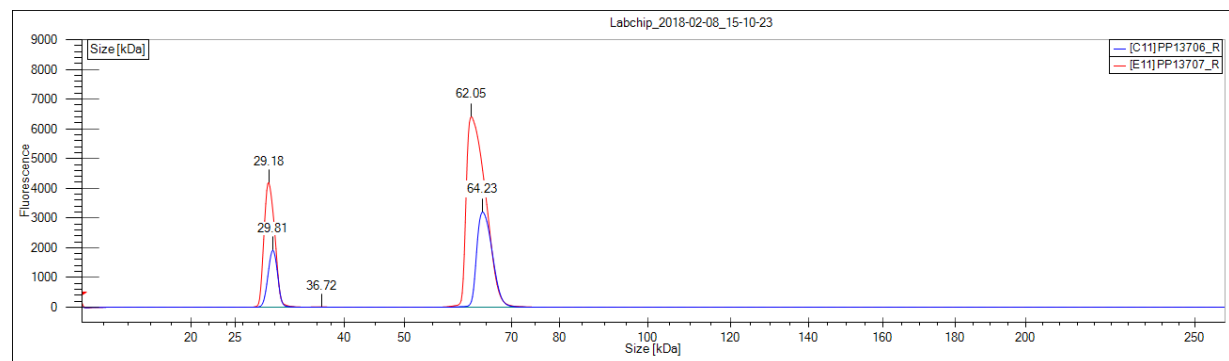
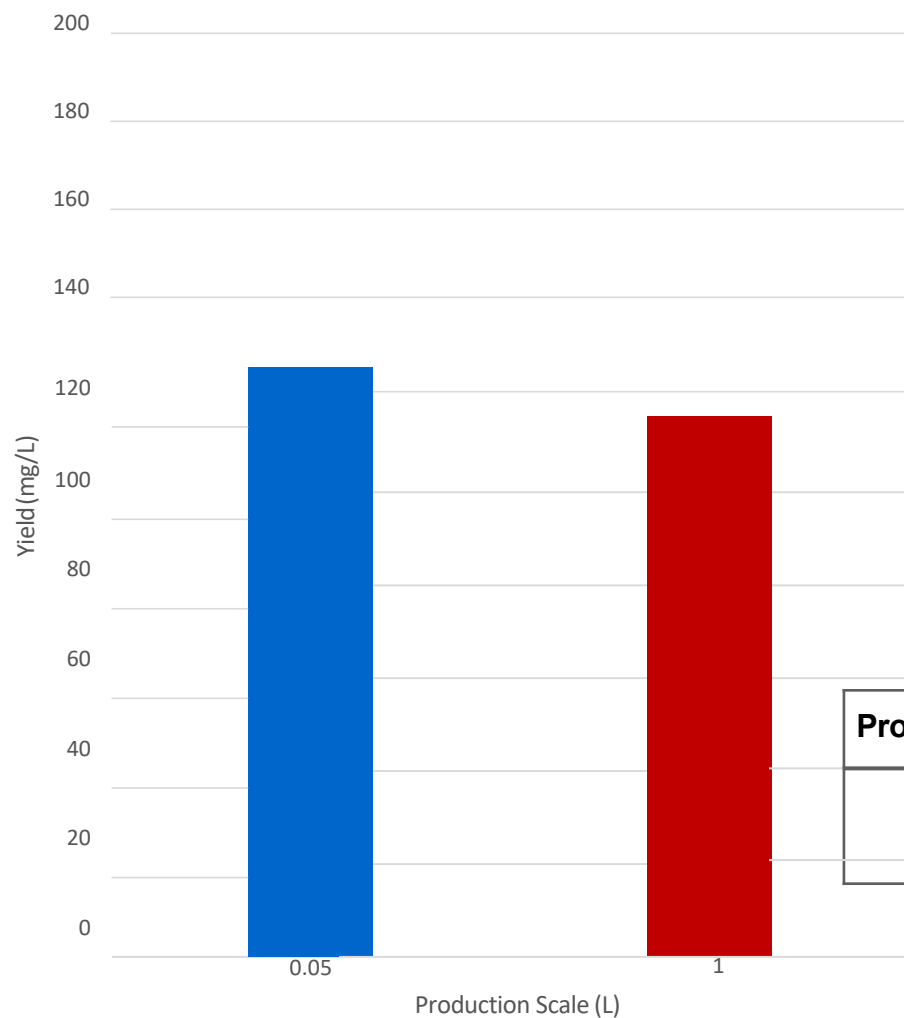


Data from extended 14-day production using TunaCHO is shown.



# 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 hlgG1 demonstrate consistency and scalability.



Protein Type	Production Volume	HC Measured	LC Measured	HC Calculated*	LC Calculated*	Δ HC	Δ LC
hlgG1	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.

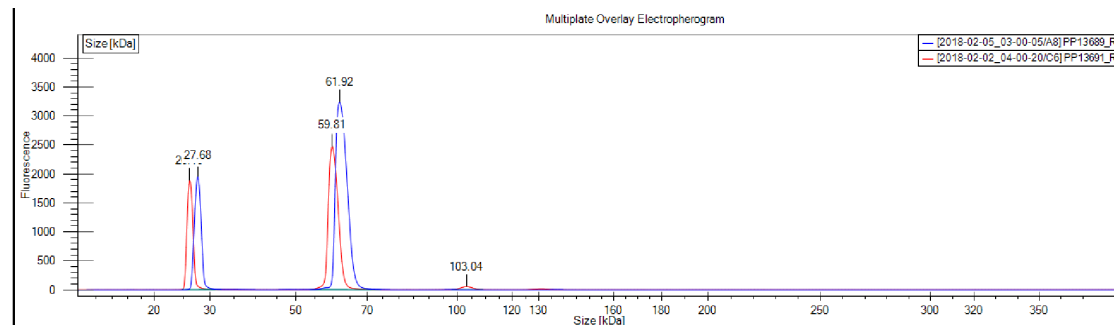
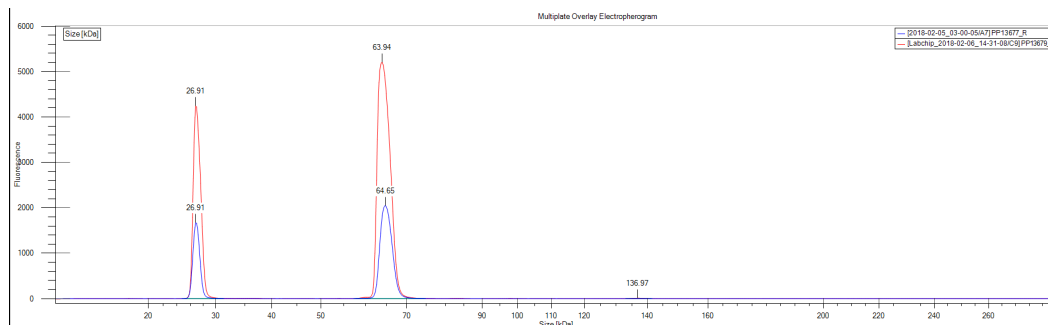
# TunaCHO- Consistent CE-SDS Profile

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

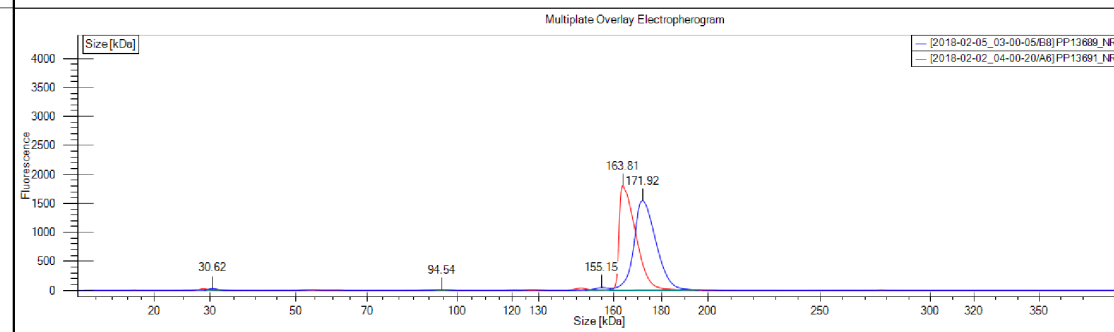
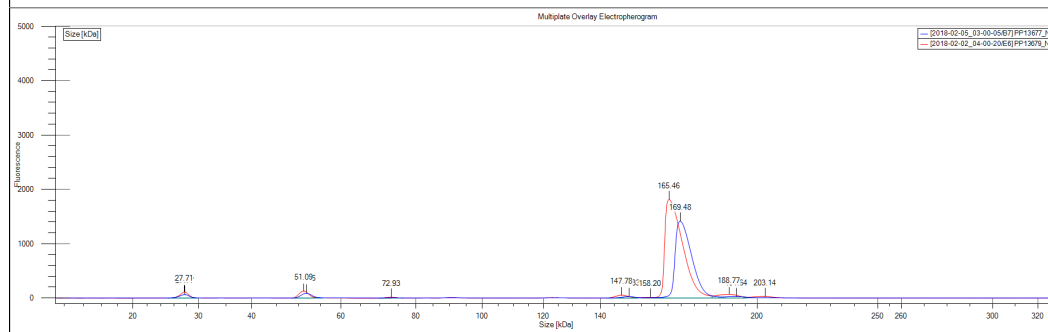
## hIgG1 #1

## hIgG1 #2

Reducing



Non-Reducing



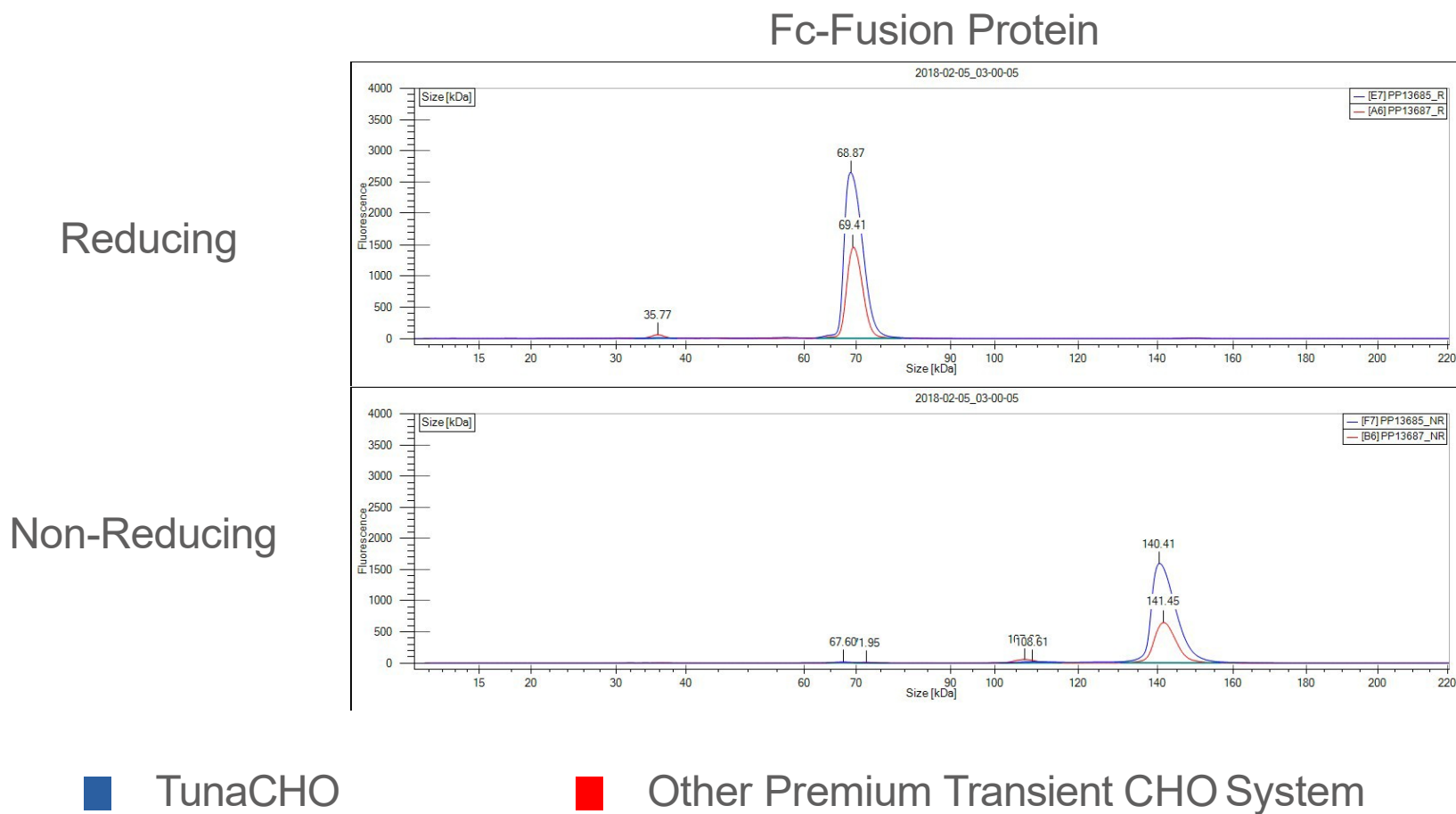
TunaCHO



Other Premium Transient CHO System

# 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.

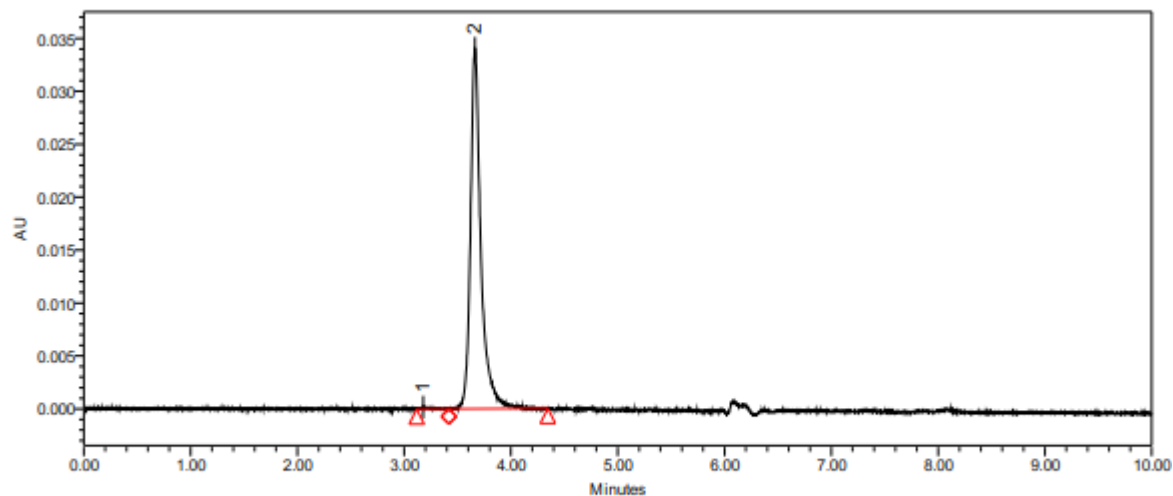


# High Purity from TunaCHO Production

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

hIgG1 #1: >600 mg/L

## Results

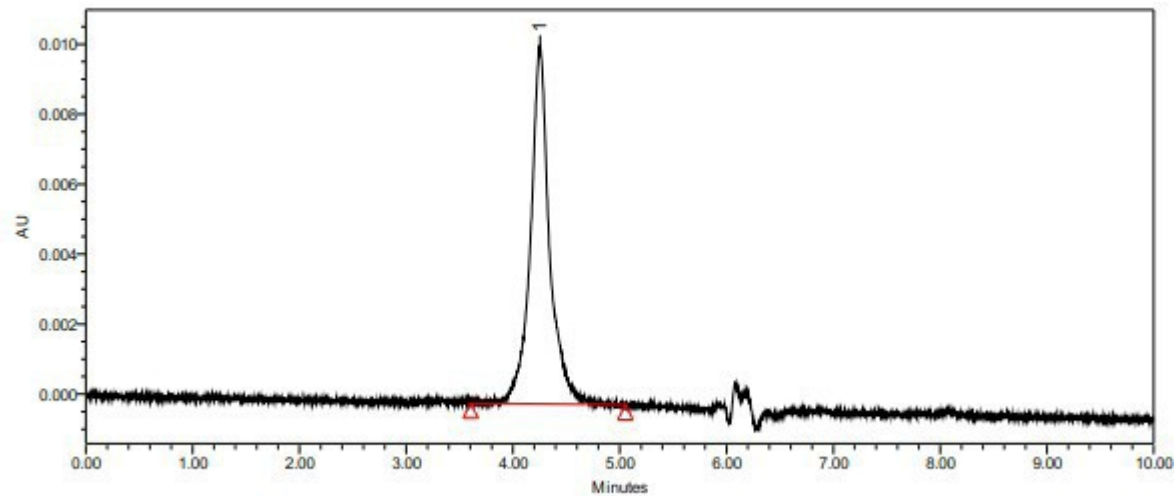


Peak Label	RT (min)	% Area	Peak Size (kDa)	Name
1	3.180	0.38	---	Aggregate
2	3.662	99.62	---	Monomer

PP13505

hIgG1 #2: >260 mg/L

## Results



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

PP13506

# 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.

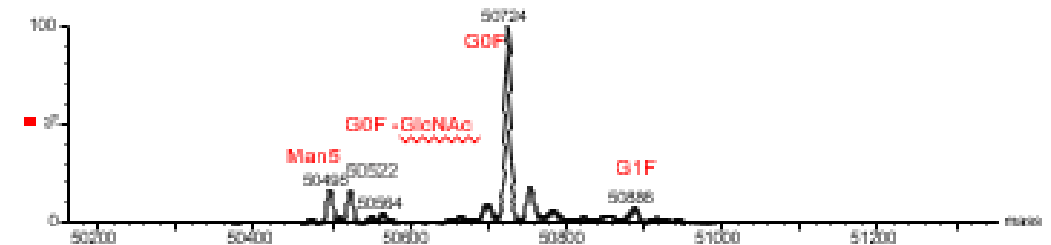
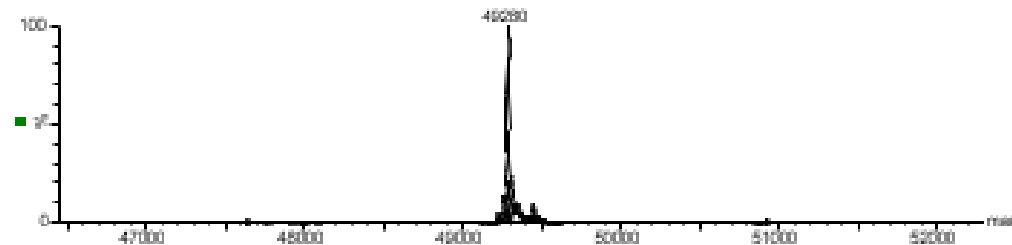
# TunaCHO - Consistent Intact Mass Results

hIgG1

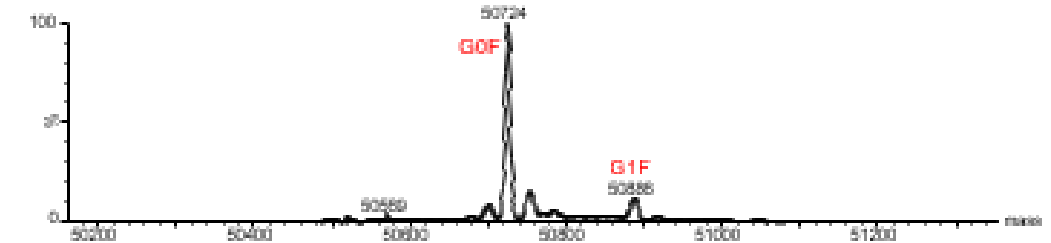
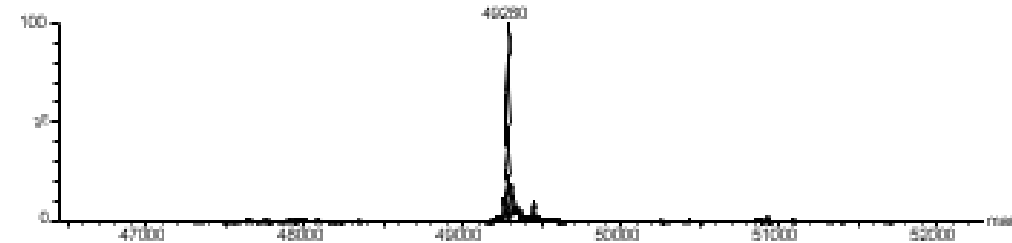
Reduced & Deglycosylated

Reduced

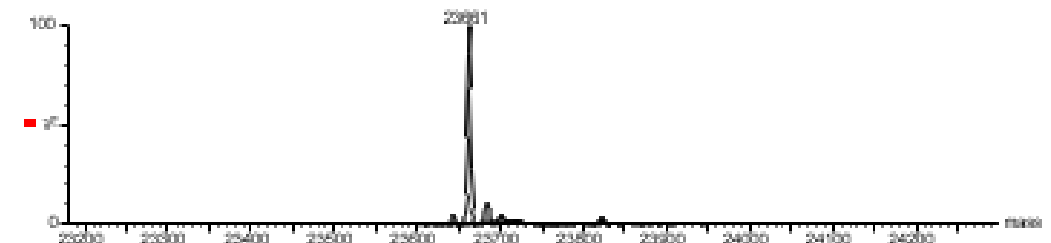
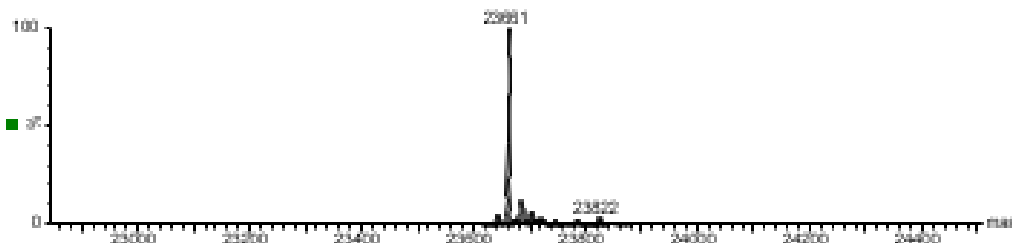
Other Premium  
CHO - HC



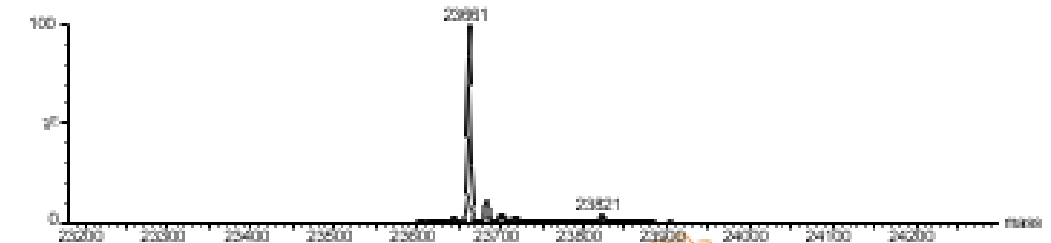
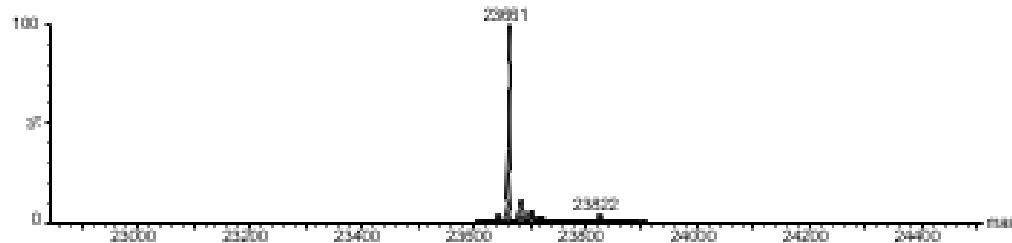
TunaCHO  
HC



Other Premium  
CHO - LC

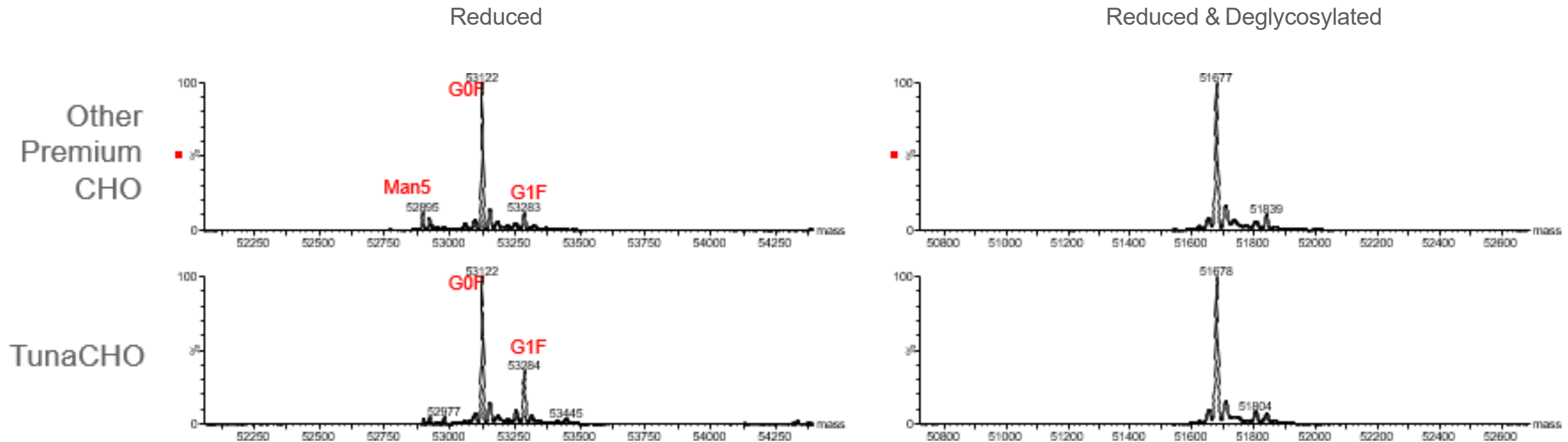


TunaCHO  
LC



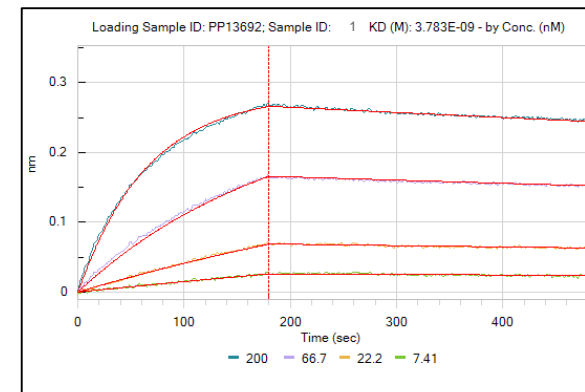
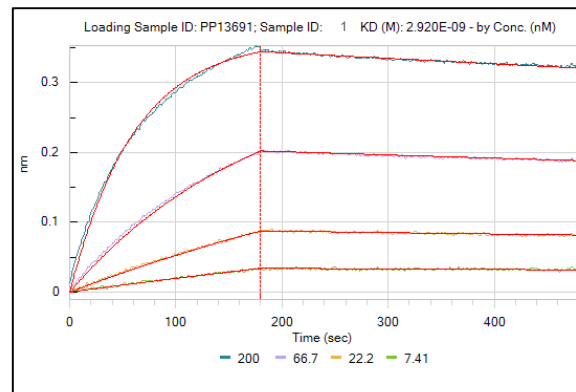
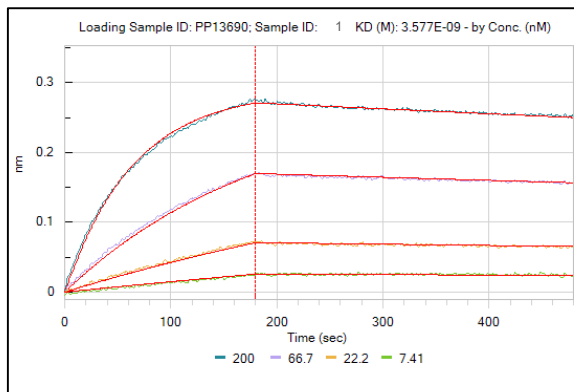
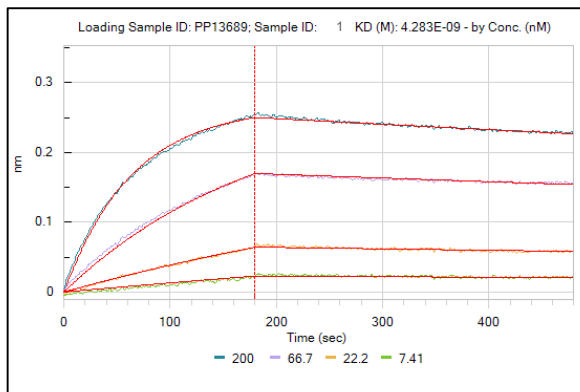
# TunaCHO - Consistent Intact Mass Results

Fc-fusion Protein



# TunaCHO - Comparable Binding Affinity

TunaCHO productions (in duplicates) were compared to another premium transient CHO system to demonstrate **comparable affinity measurement results**. hlgG1 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.



# GMP Manufacturing for ASR Using TunaCHO Platform

LakePharma can manufacture components of *in vitro* diagnostic kits for ASR or companion diagnostic applications under GMP conditions.

## Key Highlights



ISO 9001:2015 certified



Two royalty-free CHO platforms are available to accommodate different timelines and needs:

### TunaCHO Transient Platform

- Quick turnaround since RCB step is not needed
- 1 L – 100 L production tailored for specific needs

### CHO-GSN Stable Platform

- Robust, high performance
- Flexible production scale from small to large quantities



Various customization options available:

- Quantity
- Concentrations
- Release criteria
- One-time delivery or reoccurring delivery

# Available TunaCHO Transient Production Services

## 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 >](#)
- HTP production for candidate screening [Learn More >](#)
- Large scale production of control or surrogate antibodies [Learn More >](#)

### Non-Antibody Proteins Using TunaCHO

- Gene synthesis, pilot & 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)
- “Reserved Capacity Agreement (RCA)” offers accurate and reliable project schedule and timeline

For more information, please contact [Inquiries@LakePharma.com](mailto:Inquiries@LakePharma.com)