



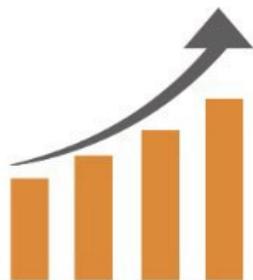
LakePharma
The Biologics Company



TunaCHO™ for Transient Protein & Antibody Production

NR5370.2020113v20

CHO Expression Systems at LakePharma

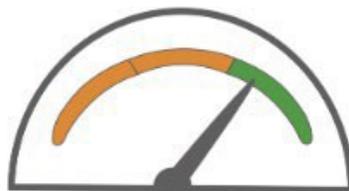


TunaCHO™

**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

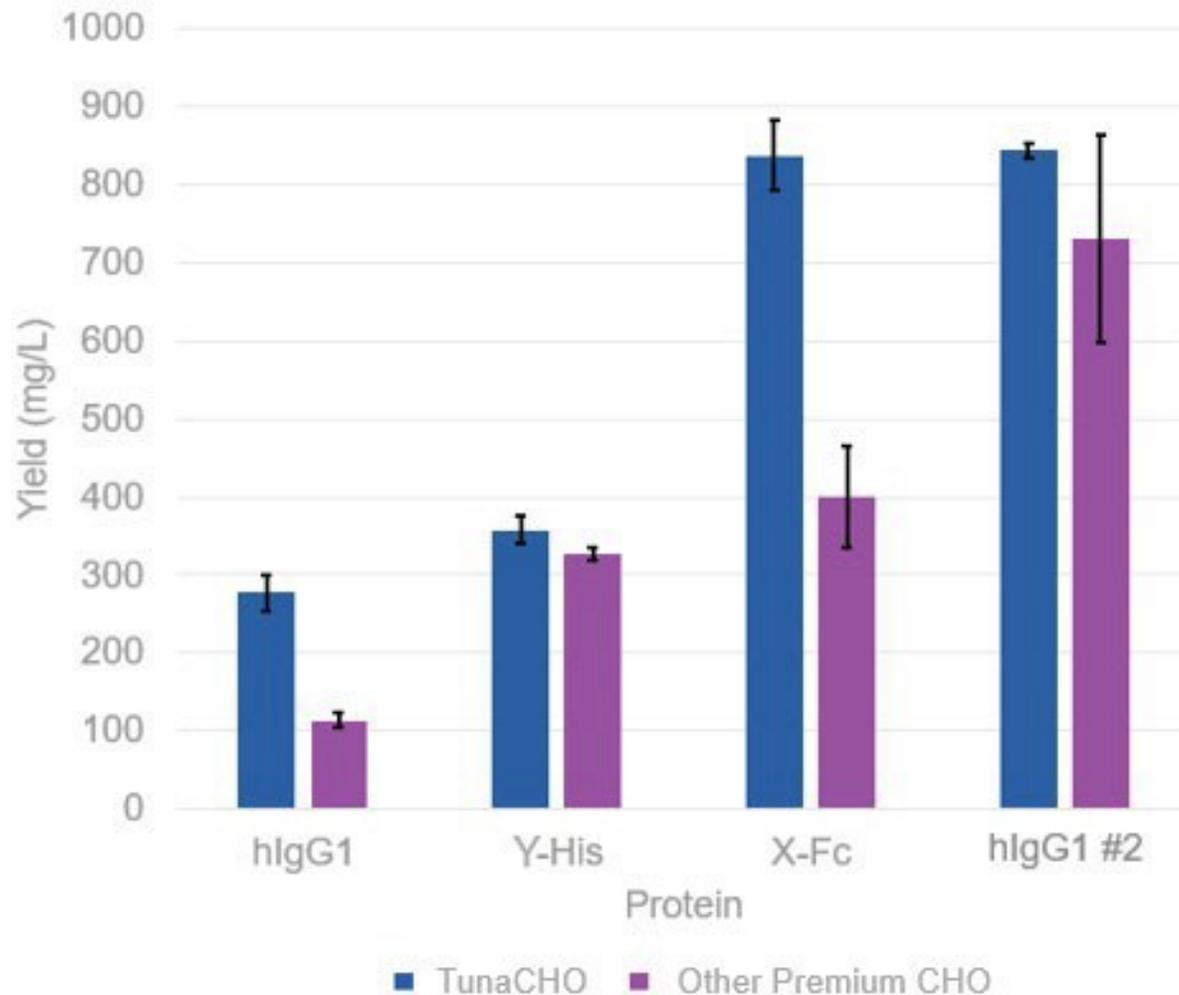
TunaCHO is a CHO transient protein production system

- 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



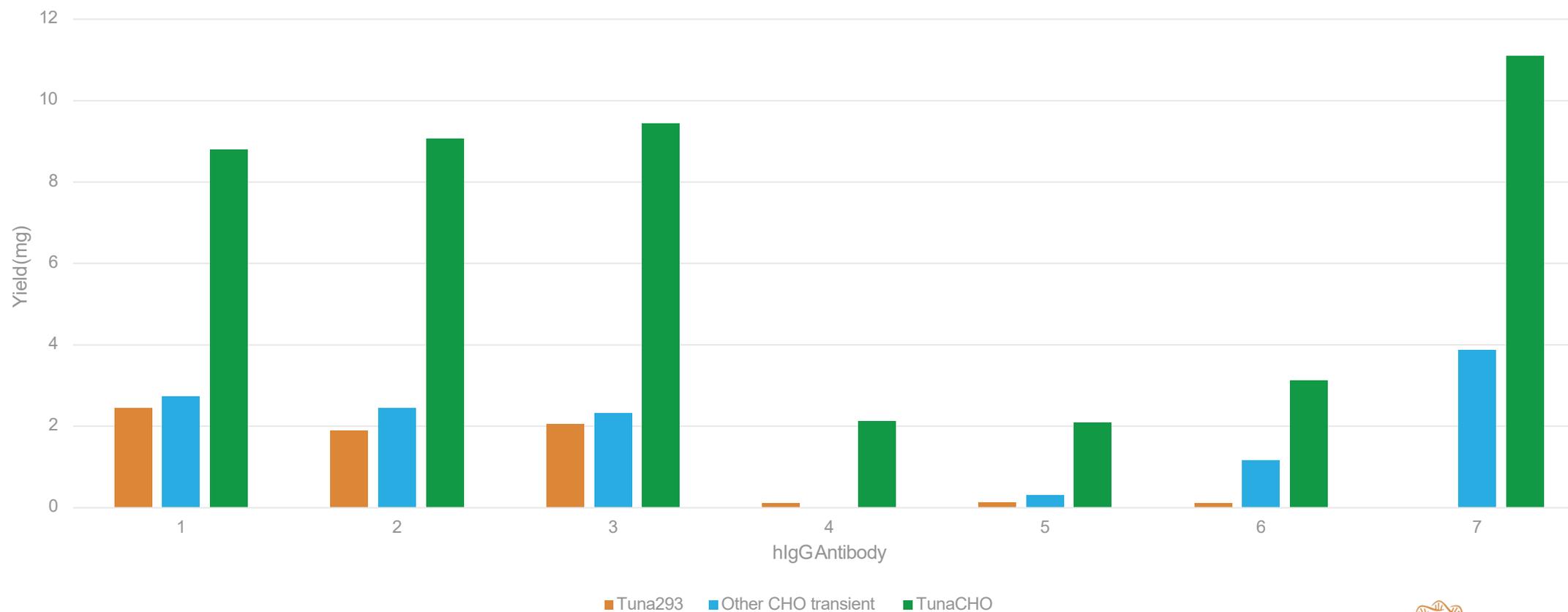
- TunaCHO as an alternative to other premium CHO transient systems in production of:
 - Recombinant human IgG1
 - Fc-fusion protein
 - His tagged non-antibody protein
- **TunaCHO process is consistent in production yield**

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.

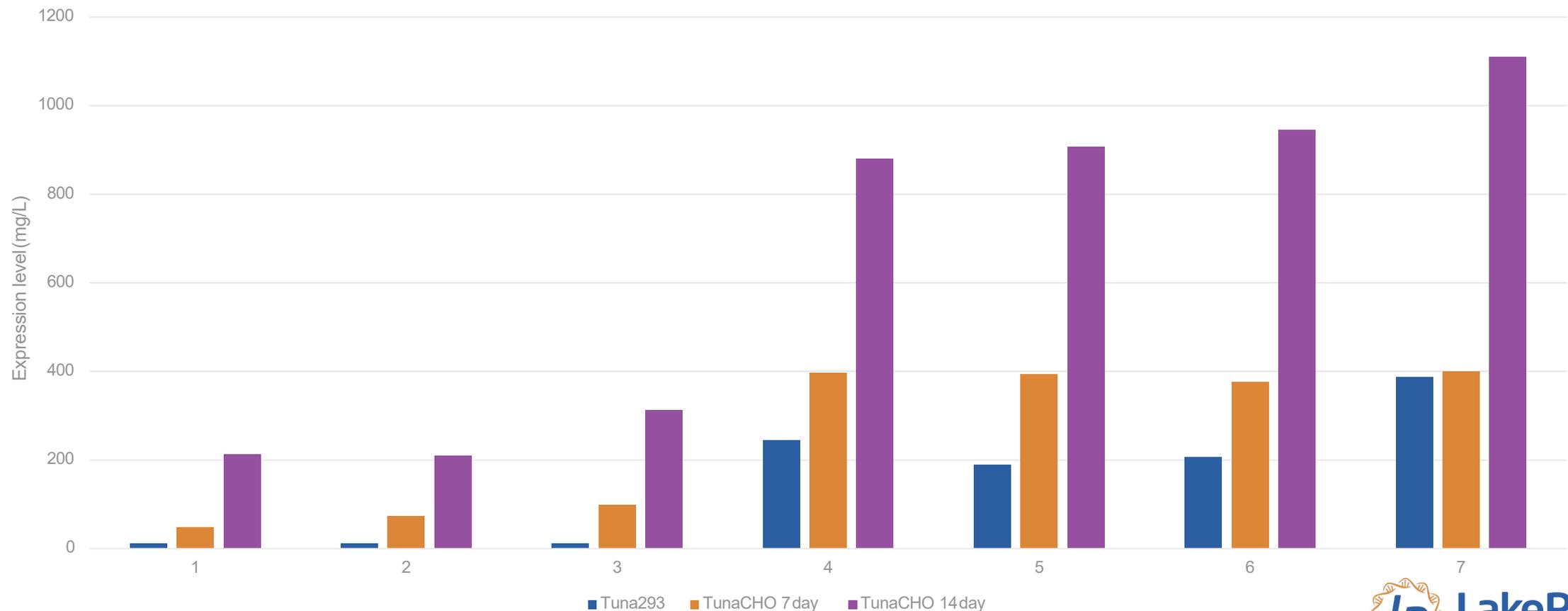


Data from extended 14-day production using TunaCHO is shown.

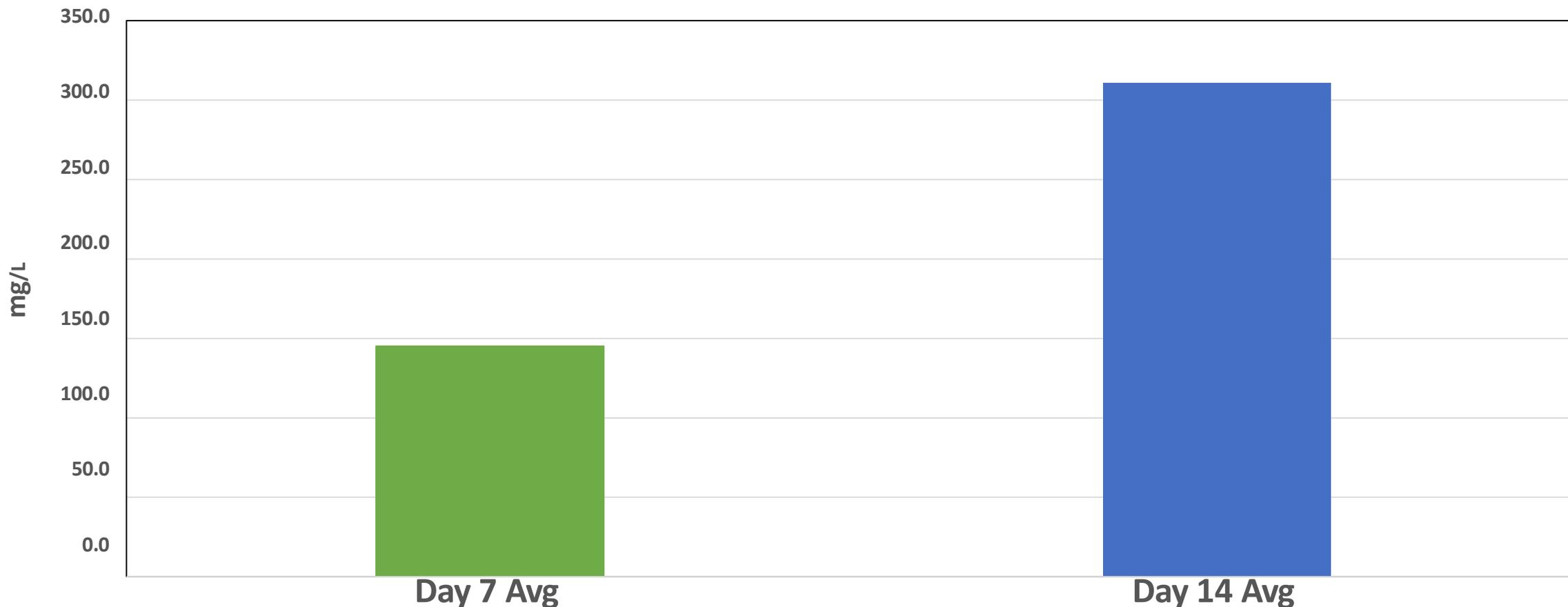
High Productivity with TunaCHO

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*

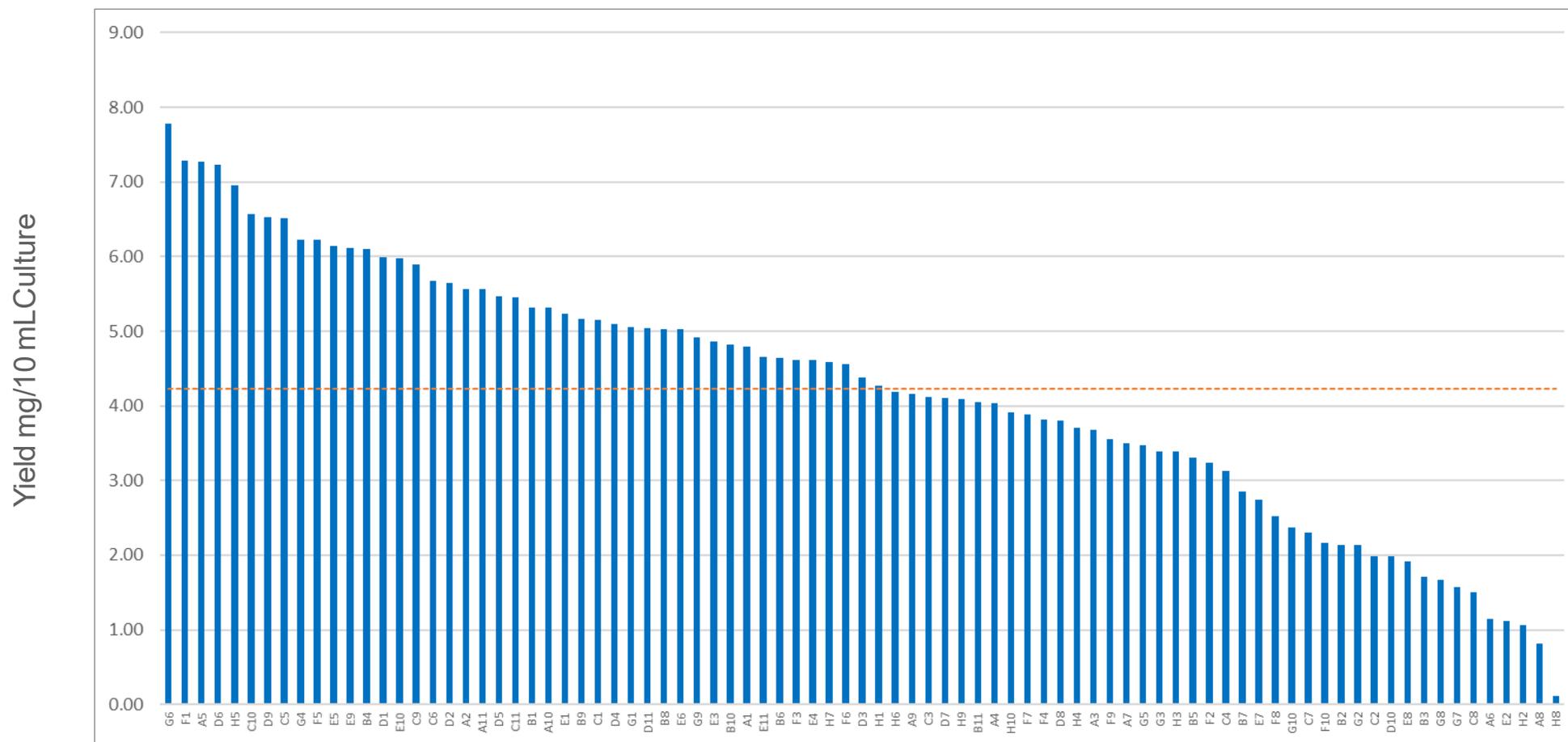


* Data from >30 production runs including human antibodies, mouse antibodies, and Fc-fusion proteins. Non-Fc proteins were not included.

TunaCHO for High-Throughput Antibody Production

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

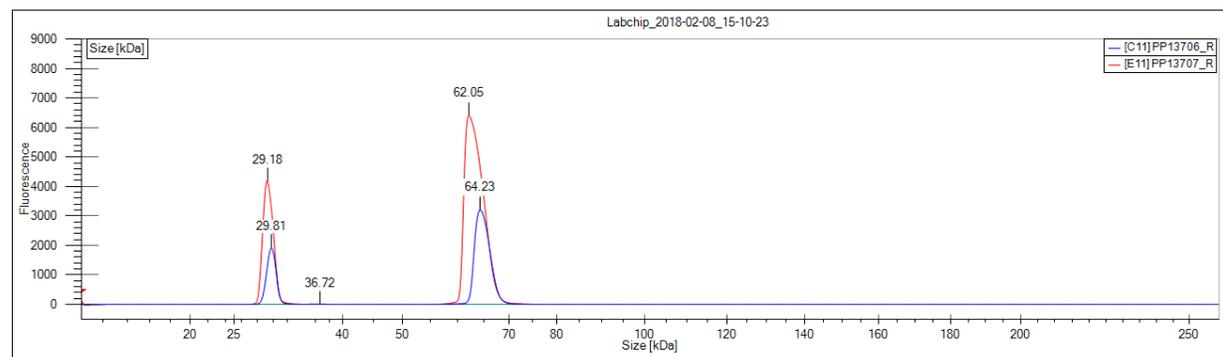
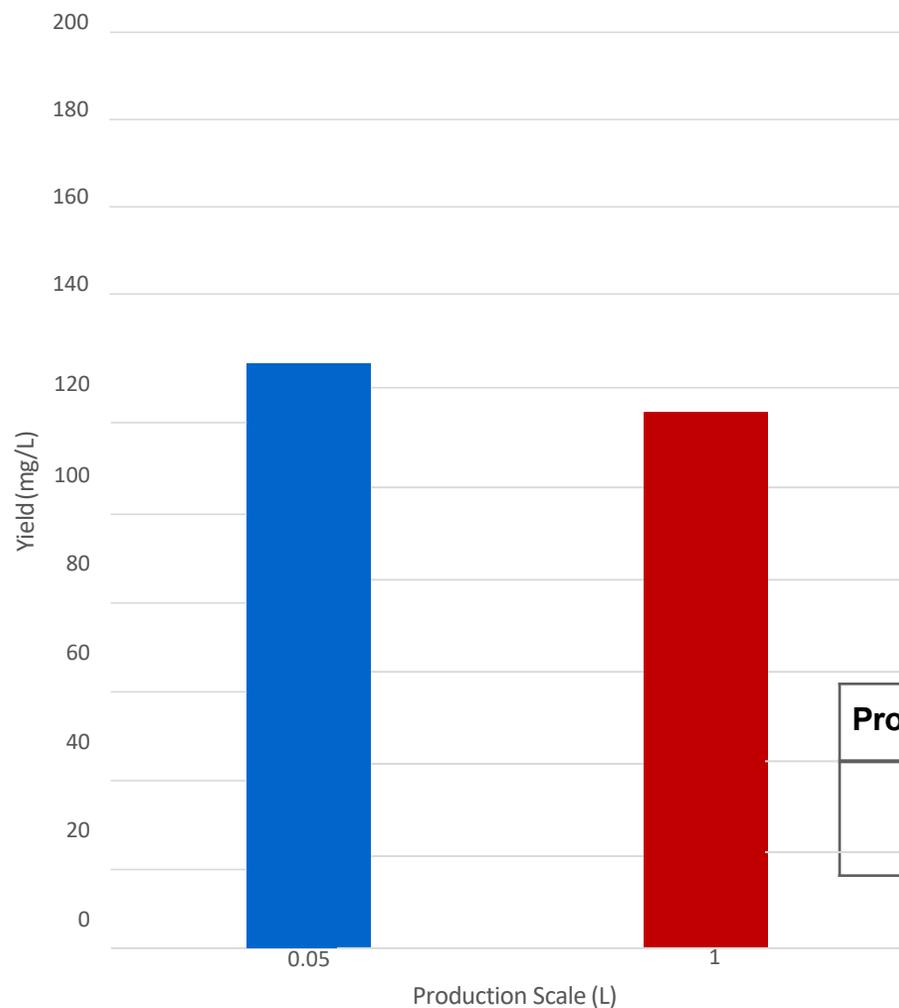
Average yield is 4.2 mg per 10 mL culture.



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.

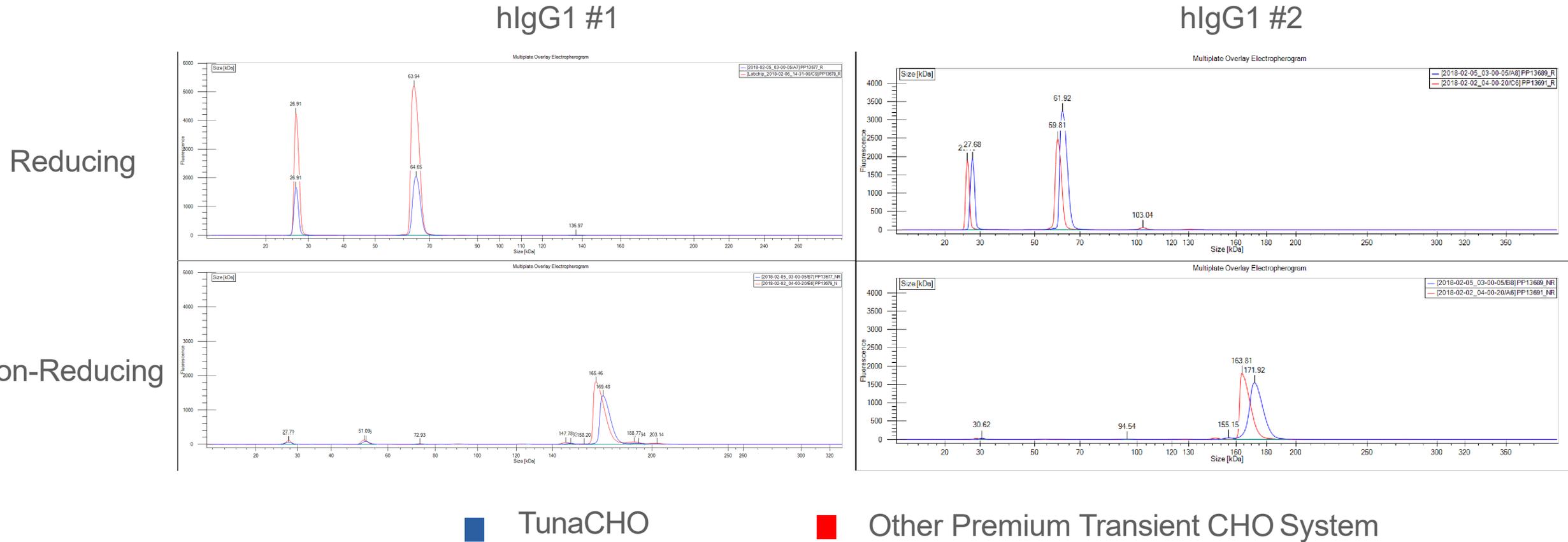


| Protein Type | Production Volume | HC Measured | LC Measured | HC Calculated* | LC Calculated* | Δ HC | Δ LC |
|--------------|-------------------|-------------|-------------|----------------|----------------|-------------|-------------|
| hlgG1 | 0.05L | 48927 | 23947 | 48925 | 23947 | 2.0 | 0.3 |
| | 1L | 48927 | 23947 | 48925 | 23947 | 2.0 | 0.3 |

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

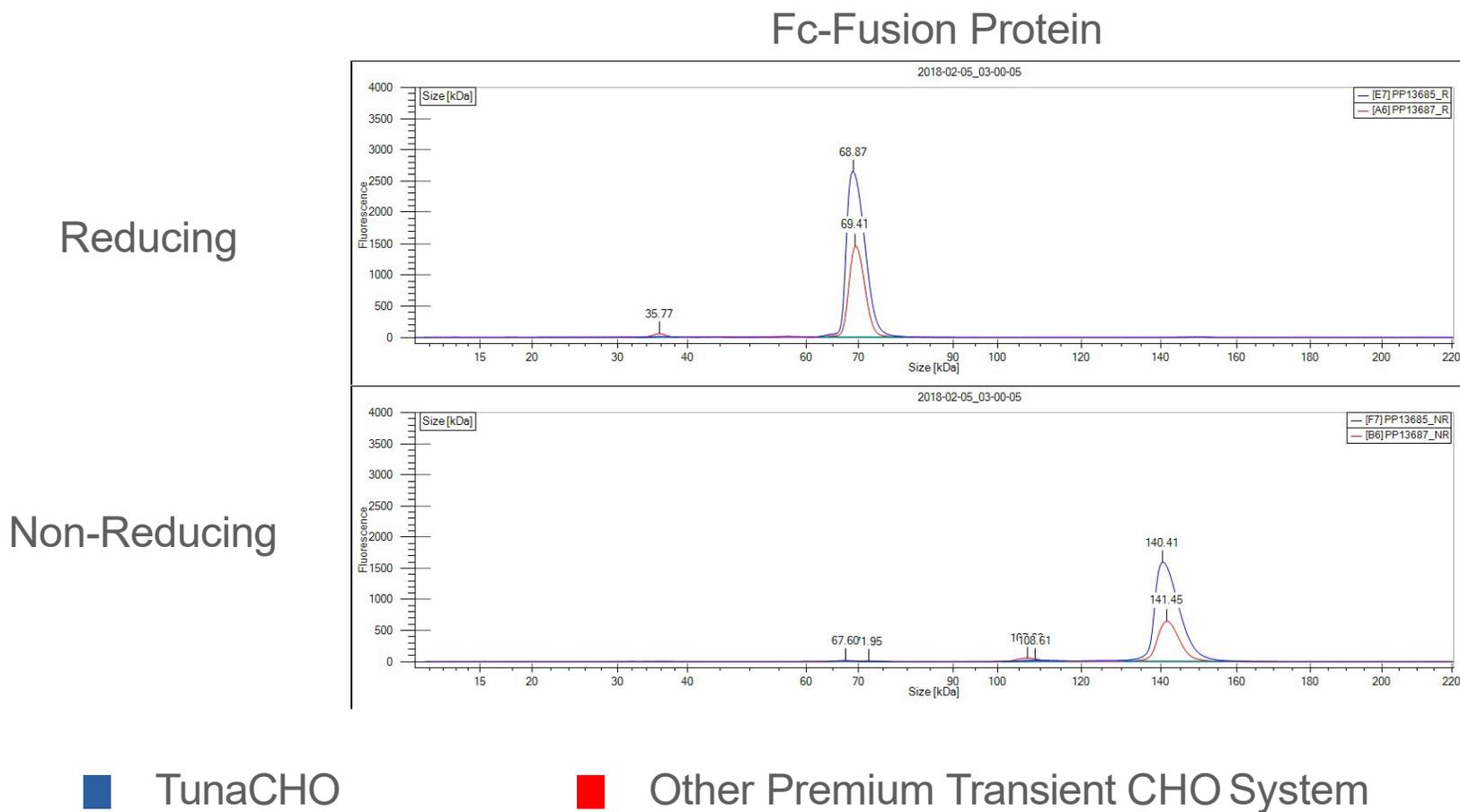
TunaCHO- Consistent CE-SDS Profile

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



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.

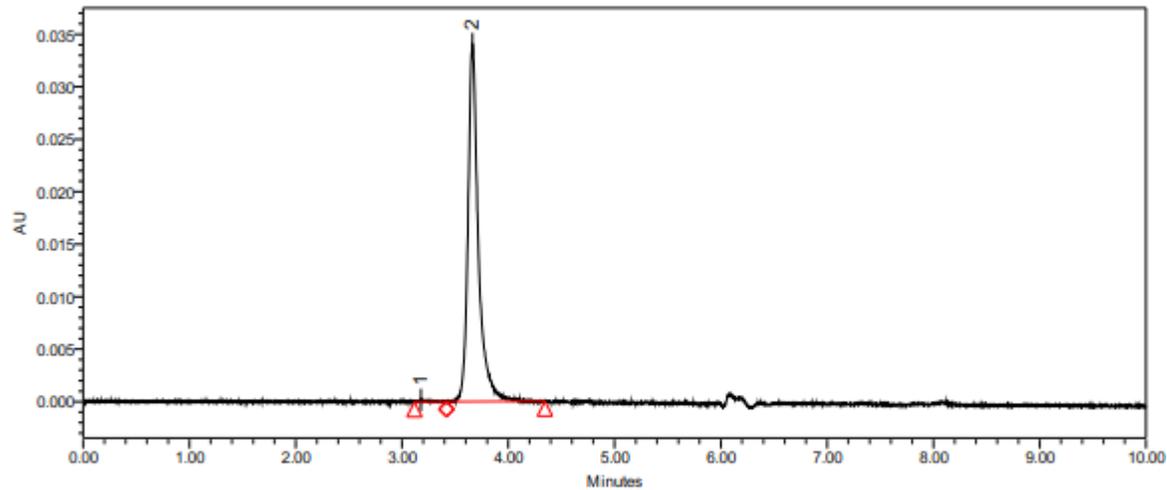


High Purity from TunaCHO Production

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

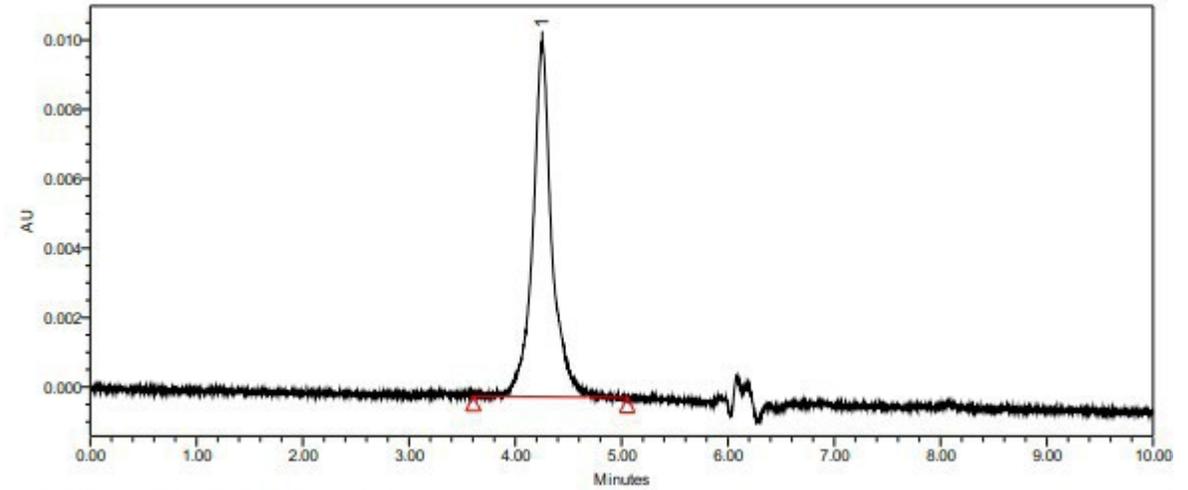


| Peak Label | RT (min) | % Area | Peak Size (kDa) | Name |
|------------|----------|--------|-----------------|-----------|
| 1 | 3.180 | 0.38 | --- | Aggregate |
| 2 | 3.662 | 99.62 | --- | Monomer |

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.

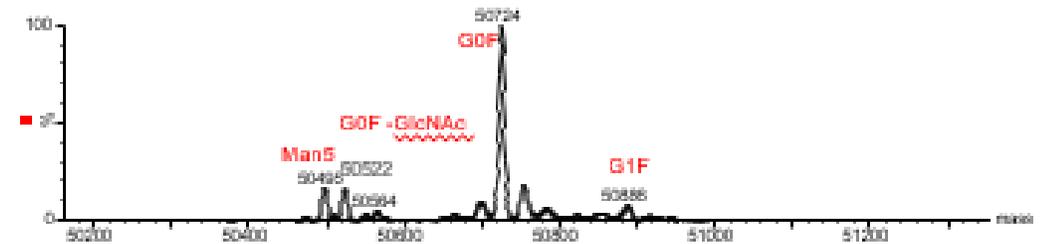
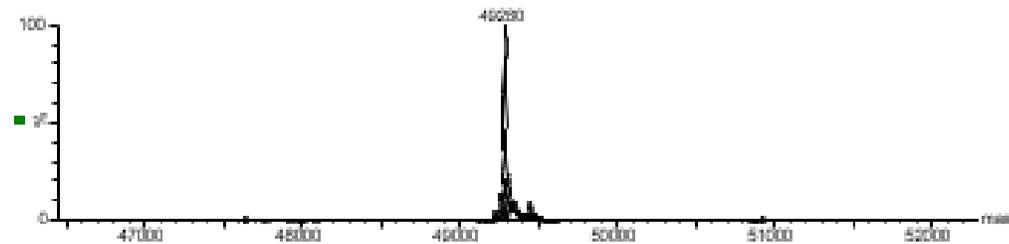
TunaCHO - Consistent Intact Mass Results

hIgG1

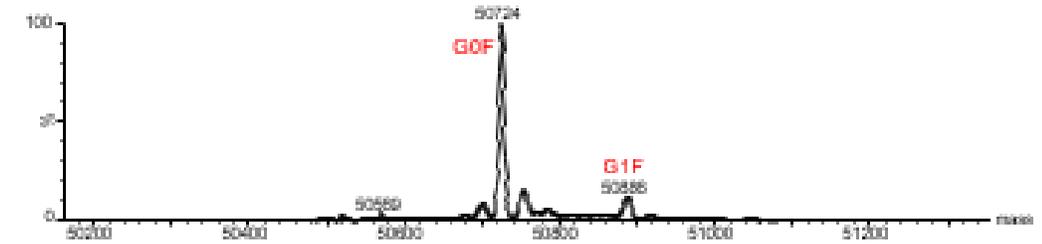
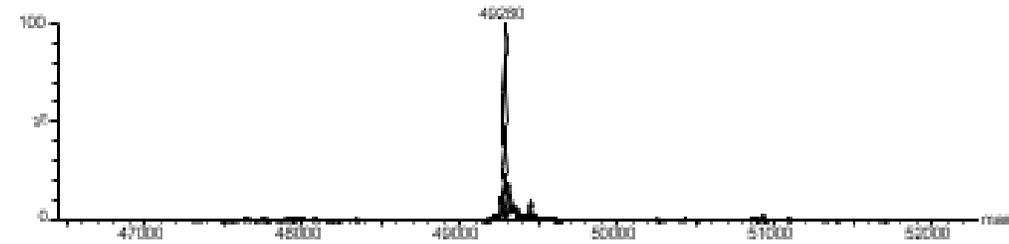
Reduced & Deglycosylated

Reduced

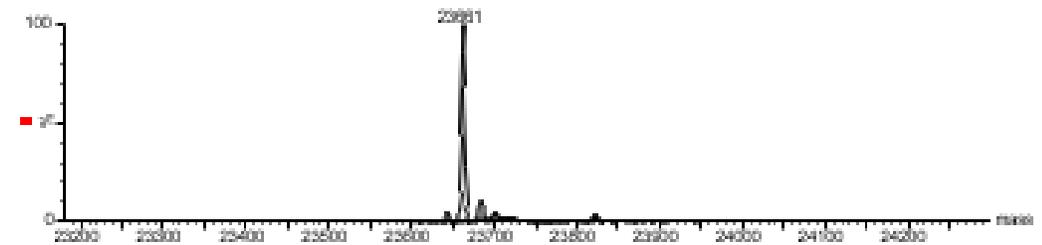
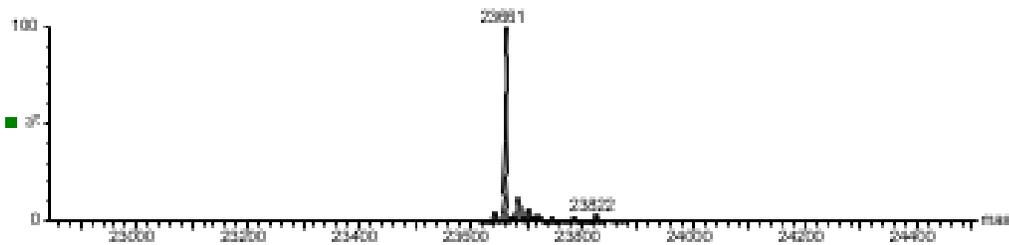
OtherPremium
CHO - HC



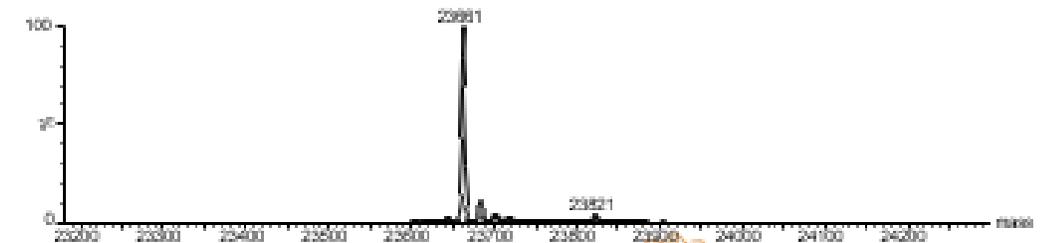
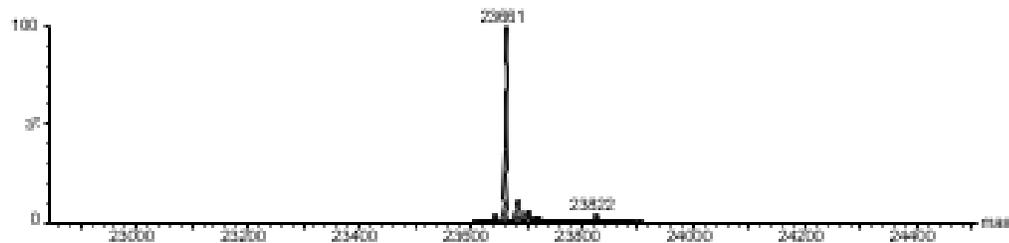
TunaCHO
HC



OtherPremium
CHO - LC

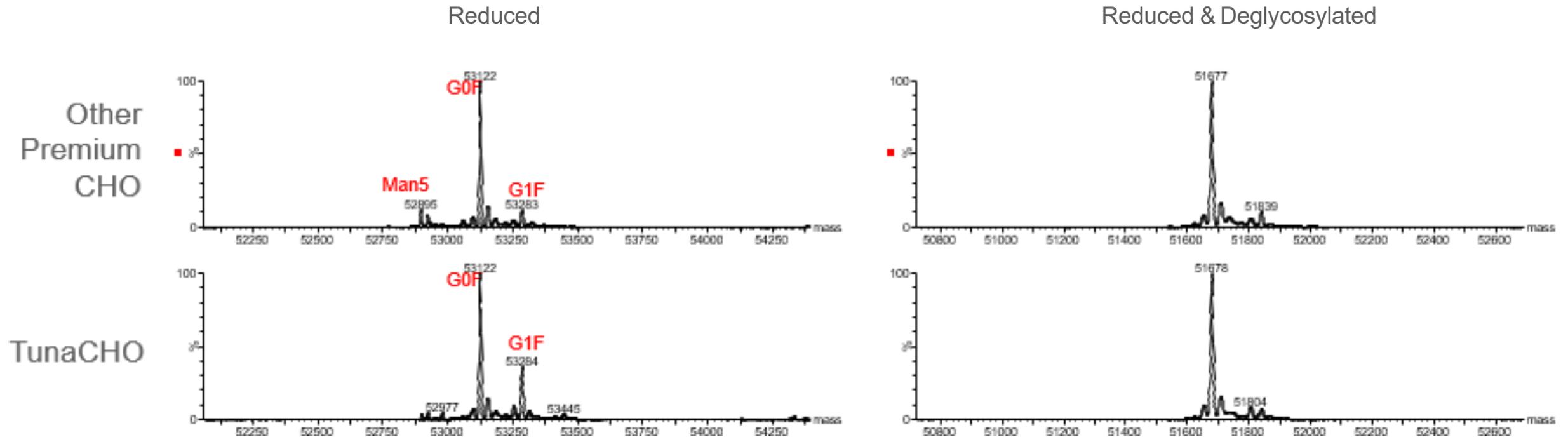


TunaCHO
LC



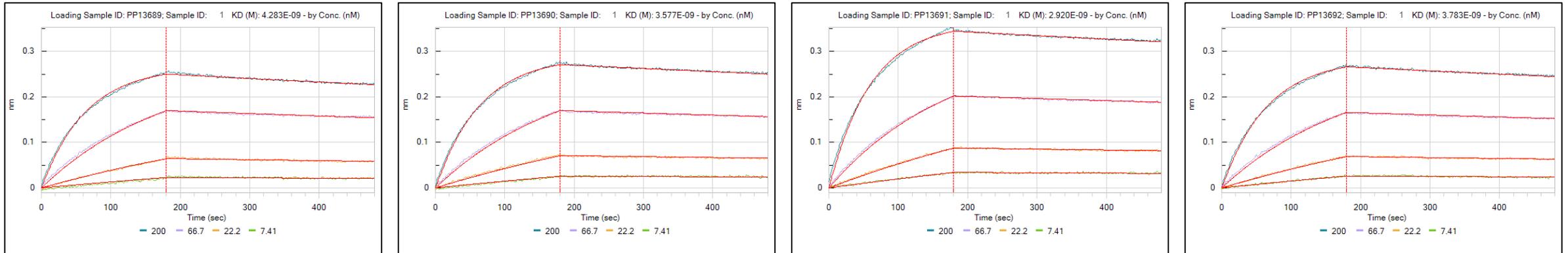
TunaCHO - Consistent Intact Mass Results

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