Analytical Innovations to Speed-up Antibody Characterization

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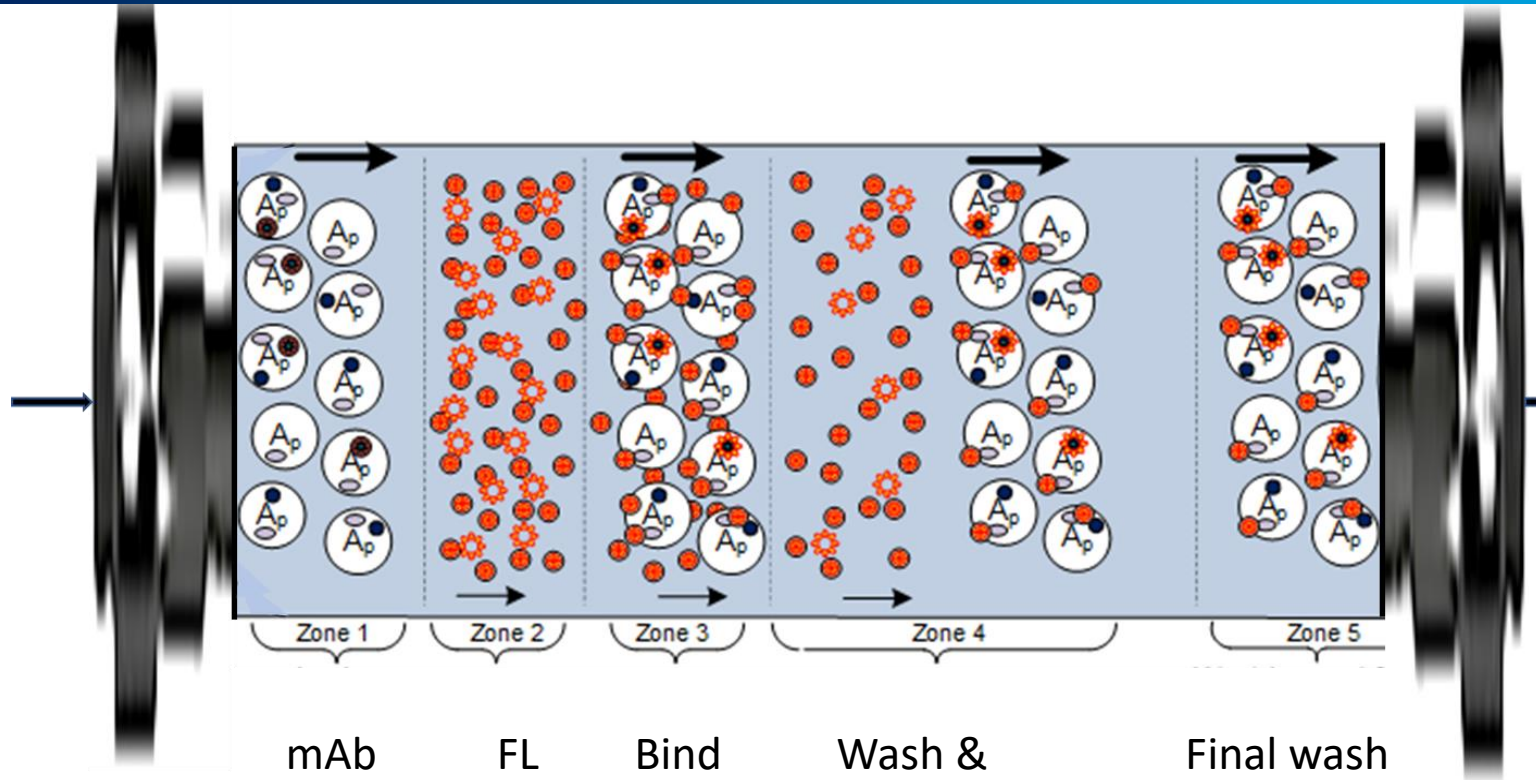
janssen
PHARMACEUTICAL COMPANIES
OF Johnson&Johnson

Outline

- Need for speed and comprehensive characterization in pharmaceutical R&D
- Part 1: **bsAb Titer and Aggregation with Mobile Affinity Selection Chromatography (MASC)**
  - Enabling Titer and Aggregation Analysis with Proteometer-L
  - Validation of bioreactor titer and aggregation using Proteometer-L
  - Rapid titer analysis
- Part 2: **Microdroplet Reactions for antibody characterization**
  - Manual syringe pump coupled with microdroplet reactions
  - Automated Flow injection analysis coupled with microdroplet reactions
  - High-Throughput Flow injection analysis coupled with microdroplet reactions

Mobile Affinity Selection Chromatography (MASC) Enables: Mix, Wash, Tag, Separate



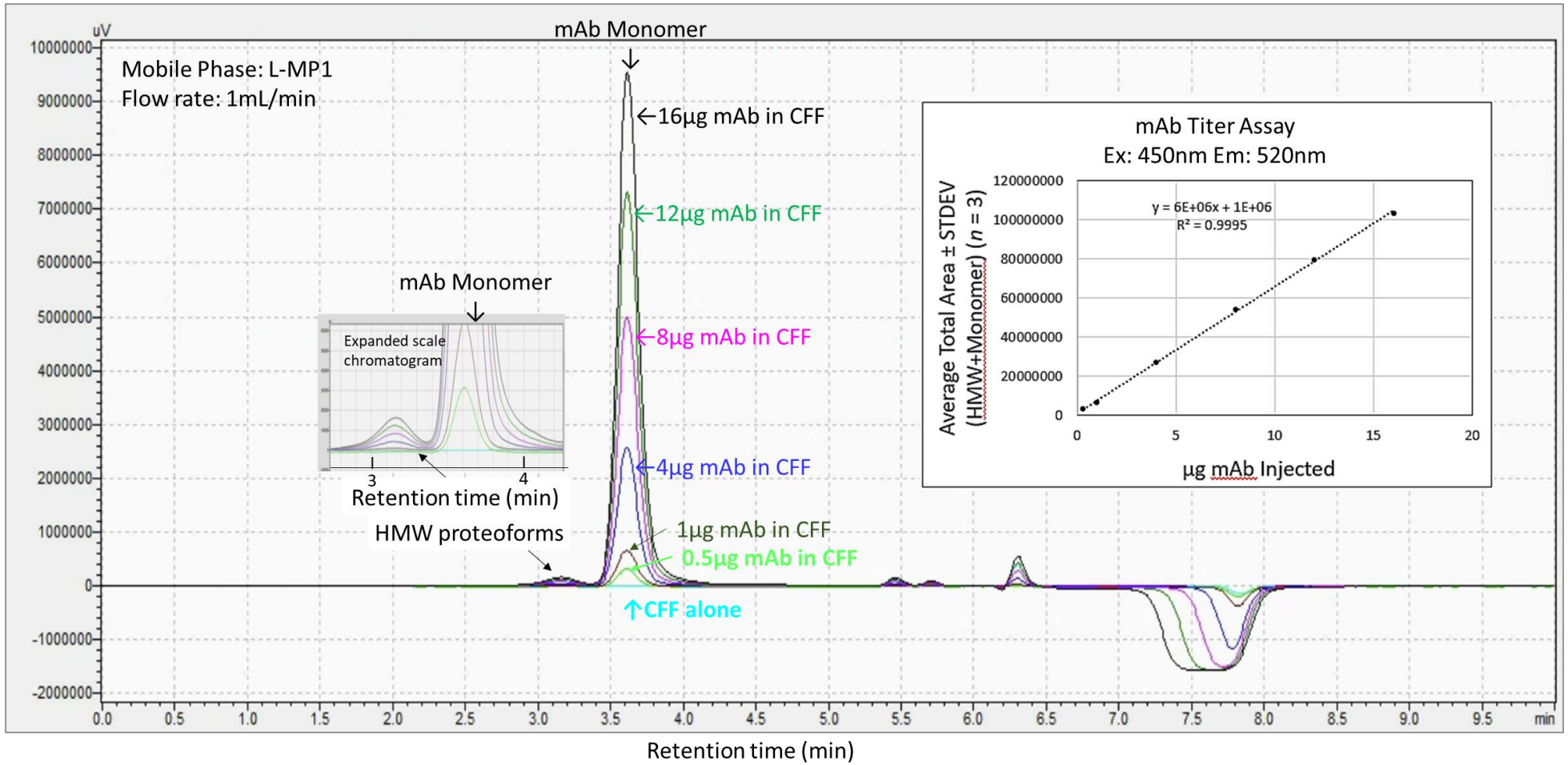
Fluorescence detection → Further structural analysis (if needed)

Note: This is a fast sandwich assay with no sample prep

Notes: (1) All the information in this presentation is Novilytic background IP, (2.) MASC, FRET assay technology, and ligand-Specific labeling of HCP's are patented technology from Novilytic, LLC (USP 10,018,635 B2 ; USP 10,670,607 B2; USP 10,065,988), (3.) At-line Reactor Test hardware and technology is Patent Pending (F.E. Regnier, et.al. Novilytic, LLC)

Proteometer-L MASC Results

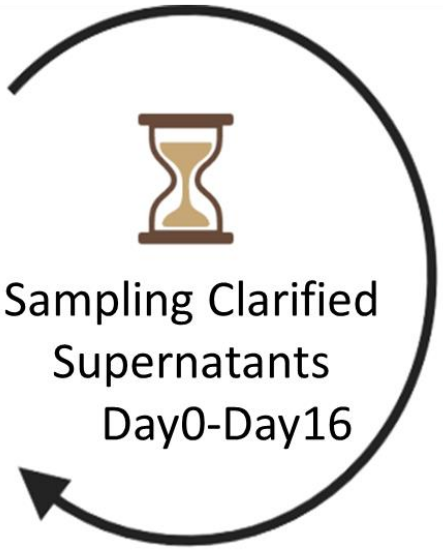
mAb Titer and Aggregate content in Crude Cell Culture Filtrate (CFF)

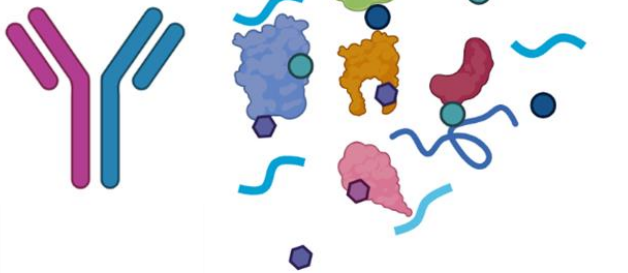


What's Missing? Answer: ~1500 coeluting host-cell proteins in the CCF

MASC Workflow to Monitor bsAb Titer and Aggregation

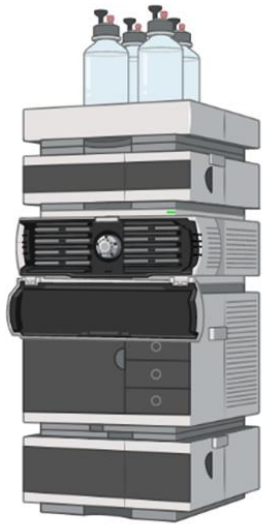
Ambr 15
Bioreactor





bsAb with CHO Cell
Proteome and
Metabolome

Luminon™ Reagents



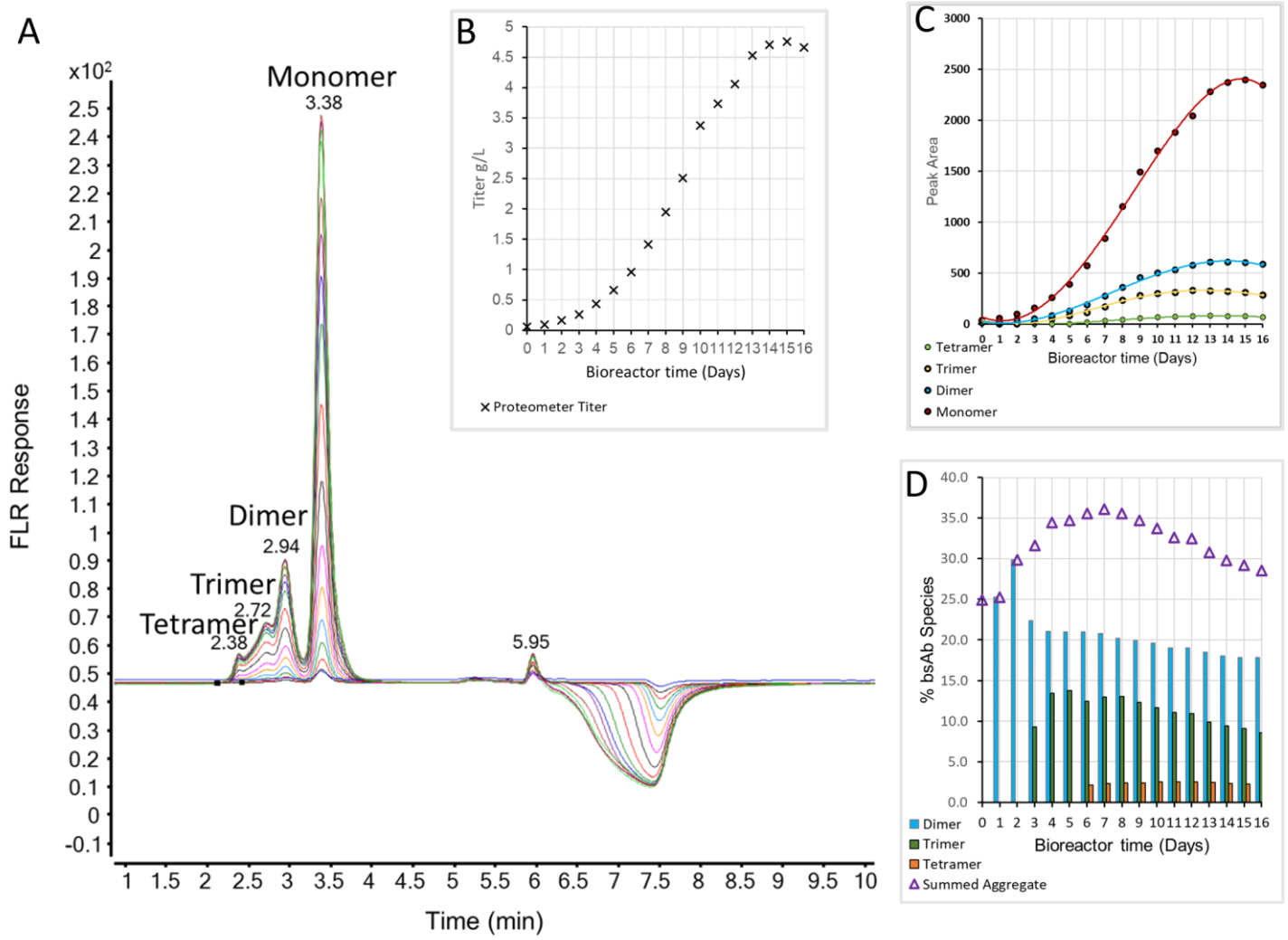
MASC

Proteometer™ Buffer System

Proteometer-L™ Column

Ambr Bioreactor time course study (Day0-Day16), of bsAb derived from CHO cells

Simultaneous Titer and Aggregation



(A) FLR response Vs. retention time of 16 CCF 16 consecutive days

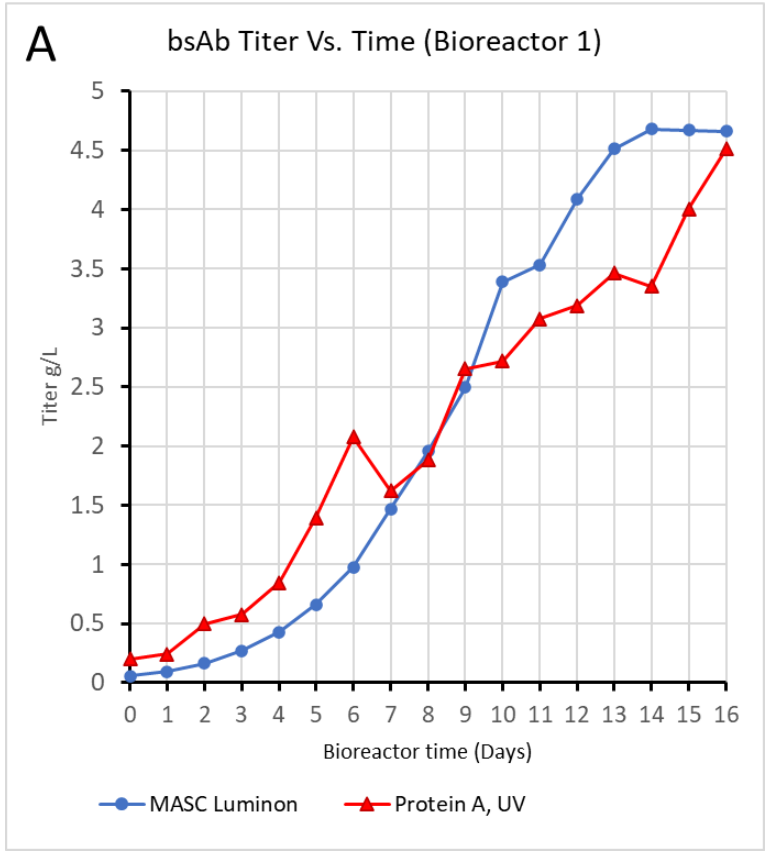
(B) bsAb titer at each bioreactor time point

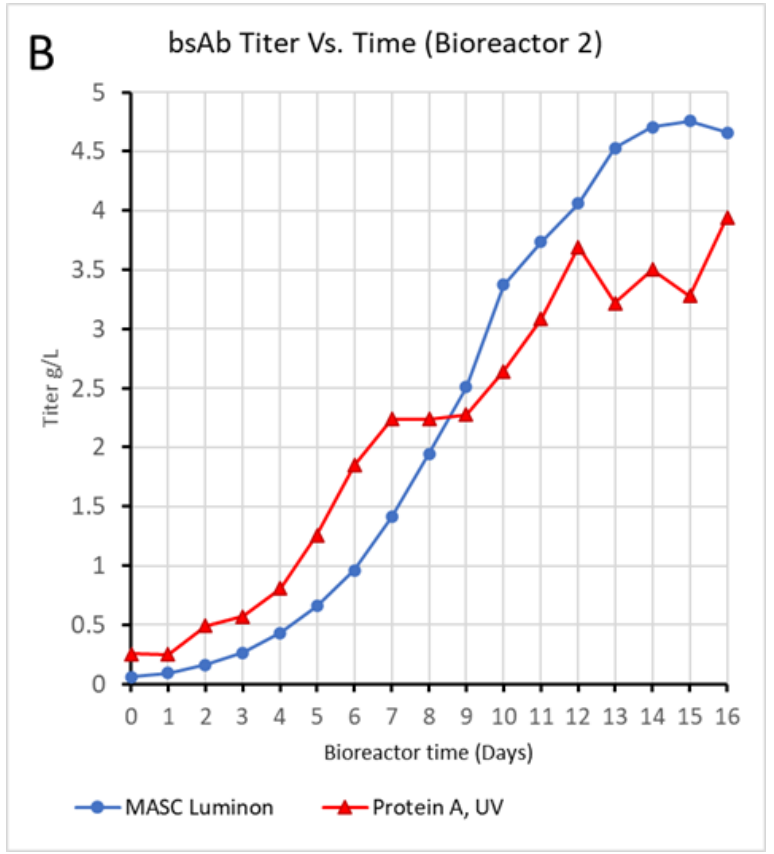
(C) bsAb-monomer, dimer, trimer and tetramer over 16 days

(D) Summed aggregation and aggregate composition profile of a bsAb

MASC monitoring of a therapeutic bispecific antibody (bsAb) titer and aggregation over the course of a single Ambr bioreactor run.

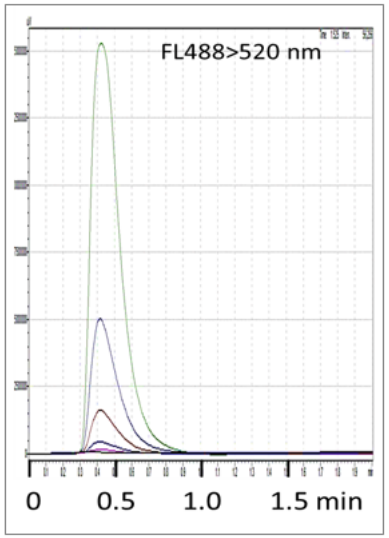
Proteometer-L Titer Reproducibility

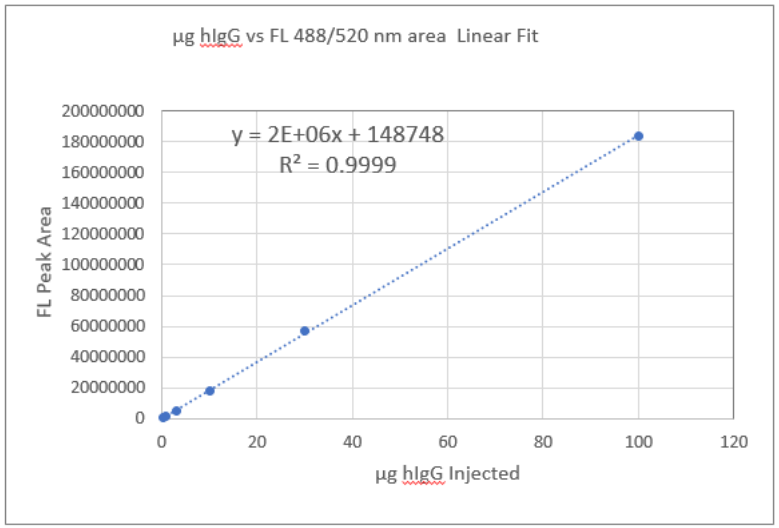


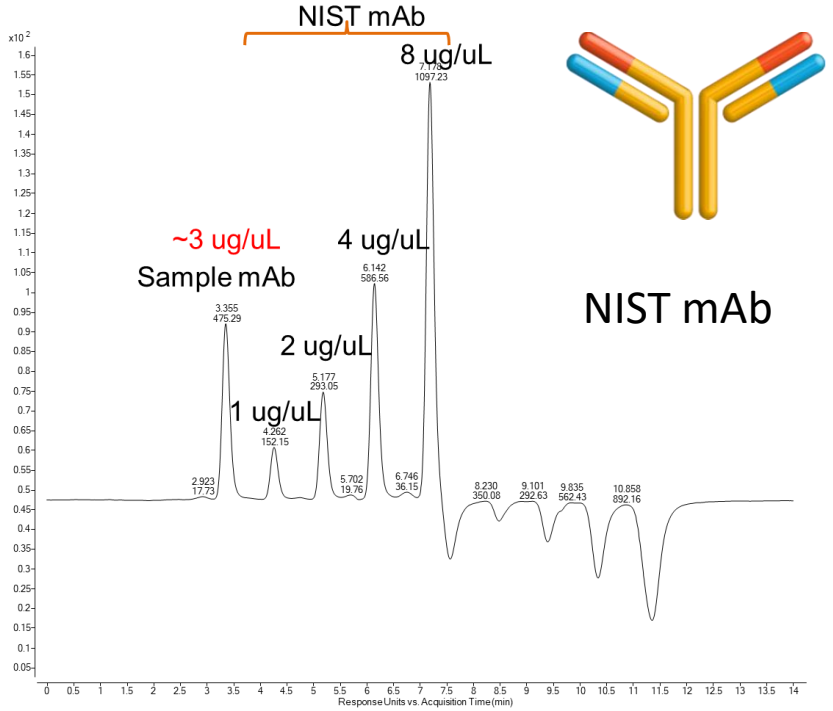


Comparison of Titer in the CFF of a therapeutic bispecific antibody (bsAb) over a 16-day period in two single-use 250 mL reaction vessels of an Ambr 250 multi-parallel bioreactor system (A) Bioreactor 1 (B) Bioreactor 2 Note: temporal profiles of Titer estimated by MASC Luminon assay (—●—) and by Protein A, followed by UV (—▲—)

Proteometer-L for Rapid Titer







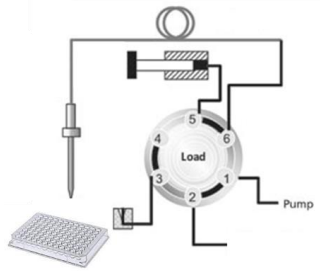
Target Applications:

1) Downstream purification efficiency measurement
2) At-line HCP confirmation

Use Injector Program

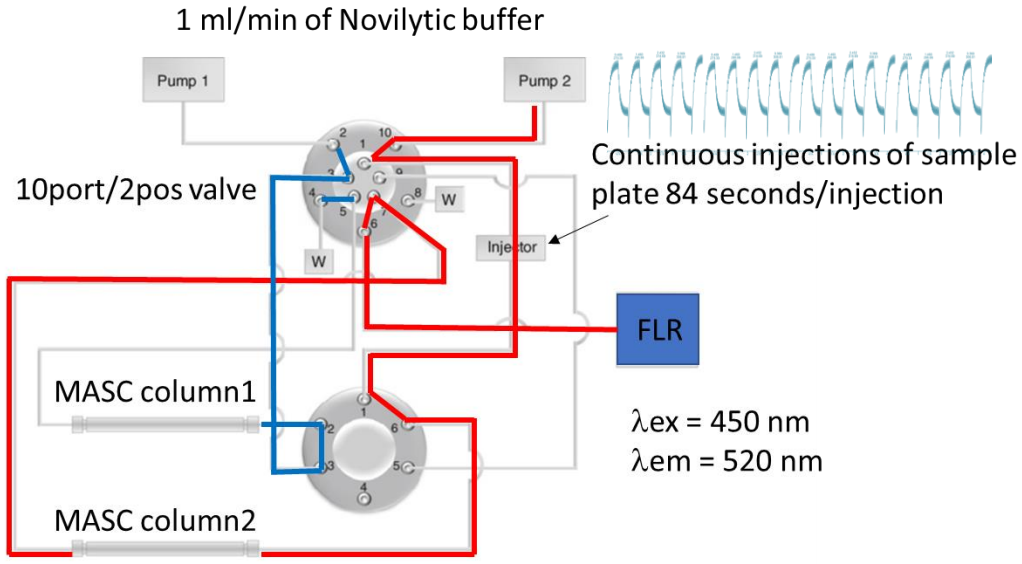
Function	Parameter
Draw	Draw default volume from sample with 50.0 µL/min using default offset
Inject	Inject
Wait	Wait 0.5 min
Draw	Draw default volume from sample with 50.0 µL/min using default offset
Wait	Wait 0.25 min
Inject	Inject
Wait	Wait 0.5 min
Draw	Draw default volume from sample with 50.0 µL/min using default offset
Wait	Wait 0.25 min
Inject	Inject
Wait	Wait 0.5 min
Draw	Draw default volume from sample with 50.0 µL/min using default offset
Wait	Wait 0.25 min
Inject	Inject

Automated Injector Script



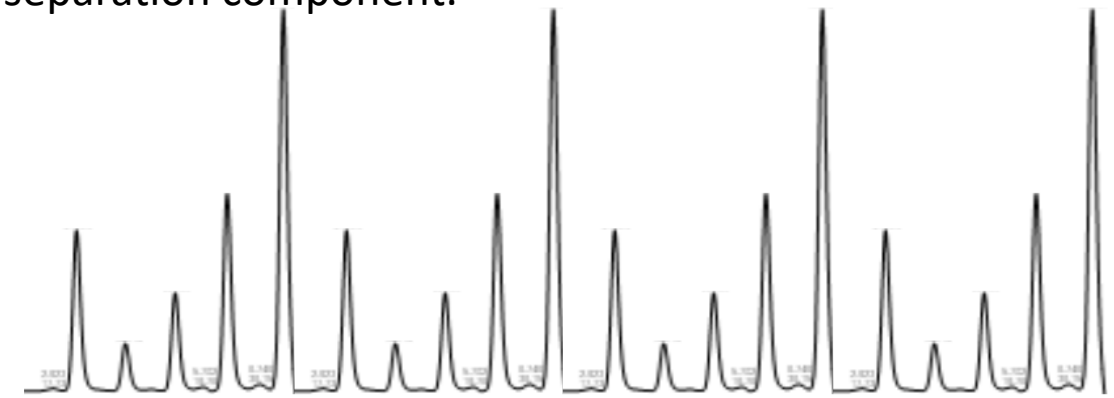
Proteometer-L- Beyond Continuous Monitoring

A dual column design is used to double the throughput of a MASC luminon assay system.



10 port and 6 port valves Triggered every 84 Seconds

The detector is seeing both monomer and aggregates. This is better than continuous monitoring because there is a separation component.



With injections being made at 1.4 min intervals (84 sec) and column switching every 7 min this platform can analyze ~40 samples per hour, i.e. ~1000 samples per day.

This is equivalent to continuous process monitoring but still allows titer and aggregation quantification plus monomer to aggregate ratio analyses at 84 second per sample.

Going a step further we can do this with antigen assays as well. This would change the process development and monitoring game completely.

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