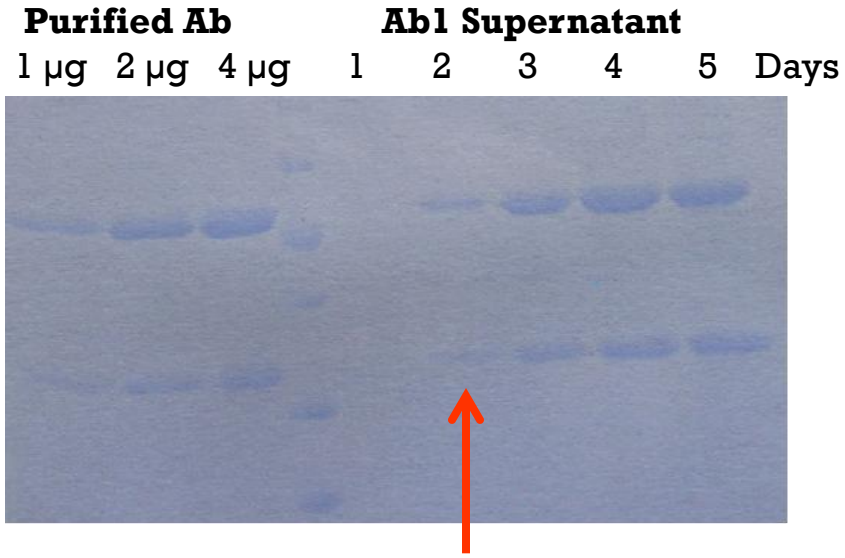
Antibody Expression in HEK293 Cells

- Chose 5 antibodies
- Variable regions cloned into a proprietary vector
- Transient transfection in HEK293 cells
- Expression in shake flasks (30 mL)
- Purification and QC per standard protocols

High Level Expression in Days

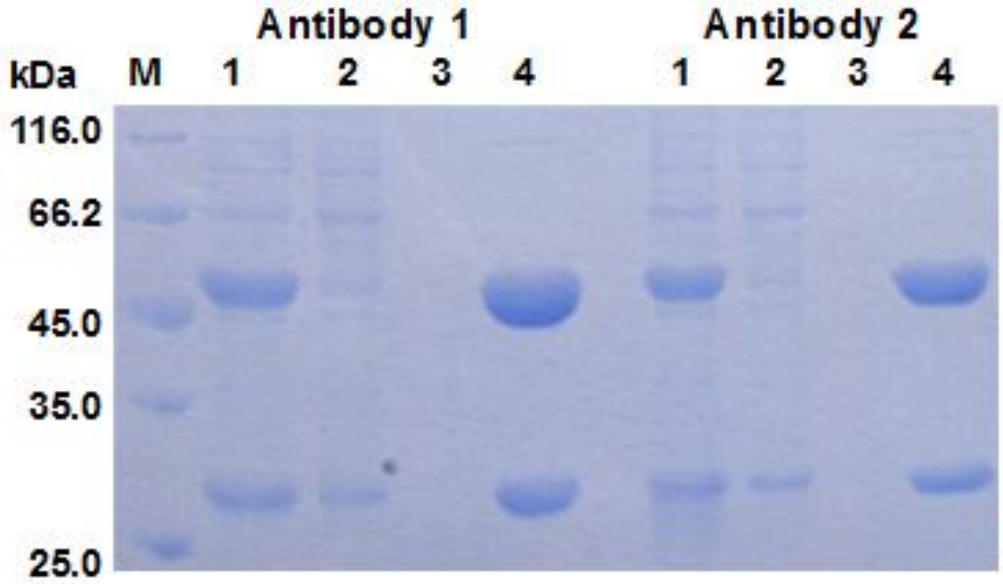


	Cell Density (x10⁶ cells/ml)	Viability (%)
Day 0	2.5	99.0
Day 1	3.8	98.4
Day 2	6.1	98.4
Day 3	5.7	93.1
Day 4	5.5	92.0
Day 5	3.7	73.0

Time course experiment shows visible Ab at Day 2

- Maximal yield on Day 4/5
- Yield increased at least **70%** by replacing media on Day 5 and growing 5 more days
- Final yield is 415 mg per liter for this Ab

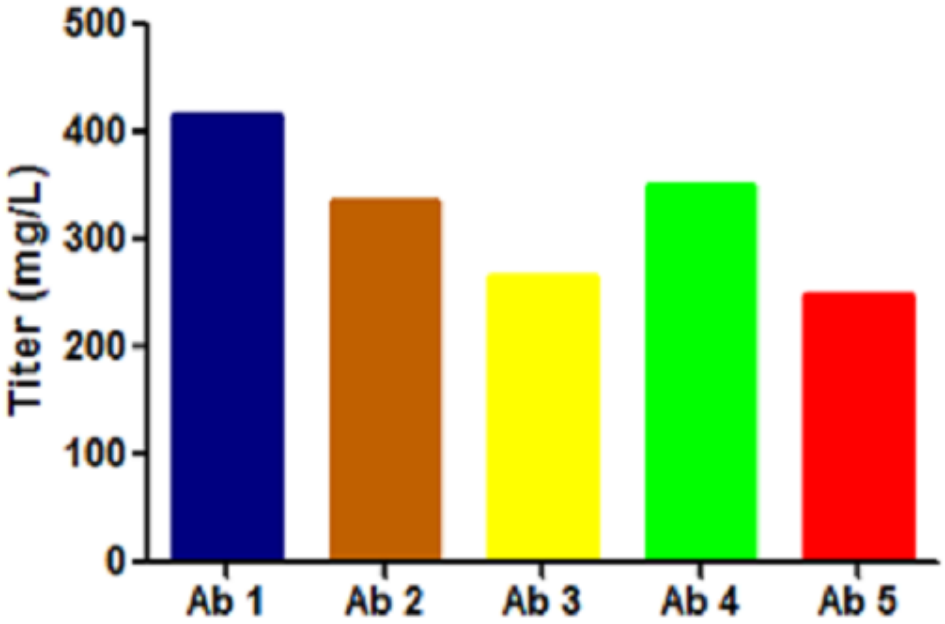
SDS PAGE Analysis



1. Supernatant
2. Flow through
3. PBS wash
4. Elution

- Protein-A resin columns were used to purify antibodies.
- Non-antibody proteins went through the columns after sample loading (2) and PBS wash (3).
- Intact antibody was eluted with the glycine elution buffer (pH3.0).

mAb Expression Titer in HEK293 Cells



- Our proprietary expression platform can deliver higher Ab yields, ranging from 247 to 415 mg per liter in shake flasks.
- The average yield is > 200 mg/L for "well-characterized" Ab.

Our titer is one of the highest in the industry!!!

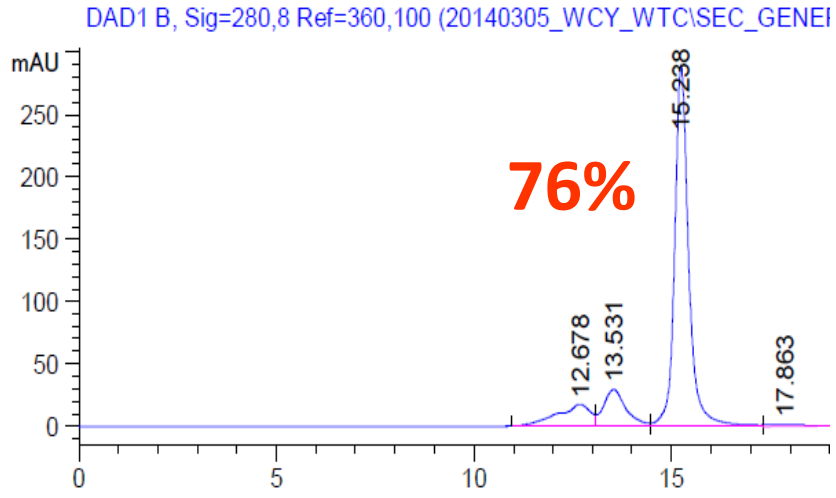
mAb Expression in CHO-S Cells

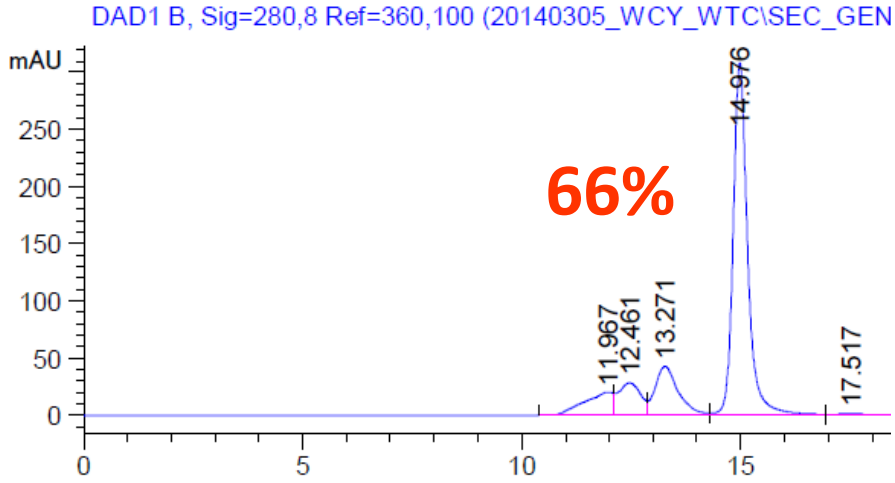
- The same single plasmid expression vector is used to express recombinant antibody in CHO-S cells.
- PEI or Freestyle MAX reagent is used for transient transfection.
- Current expression titer in CHO-S cells is ~30 mg/L.

We will continue to improve the yield in CHO-S Cells.

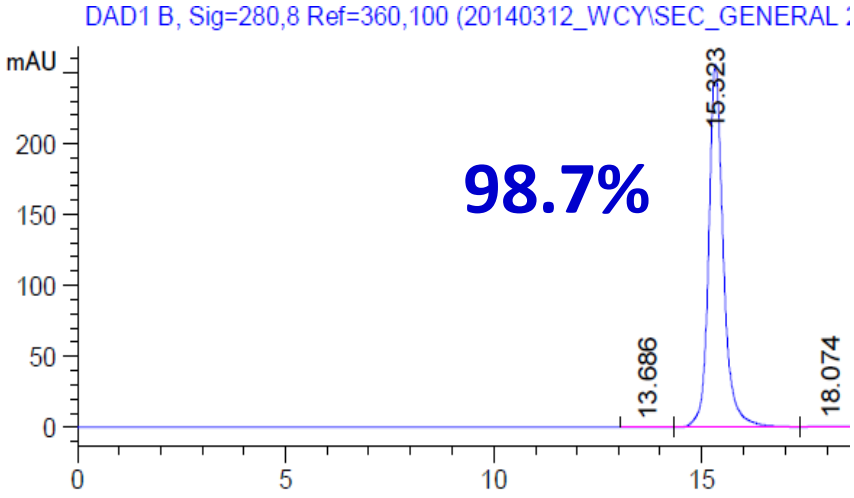
Aggregate Removal by Superdex 200

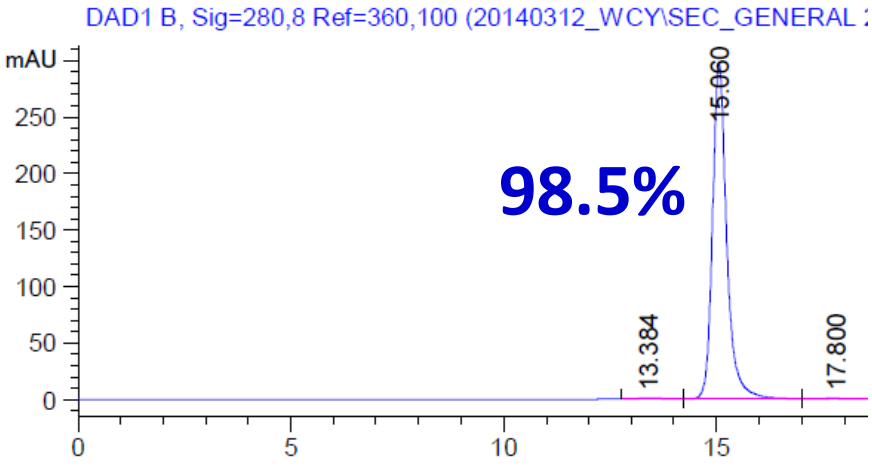
Before



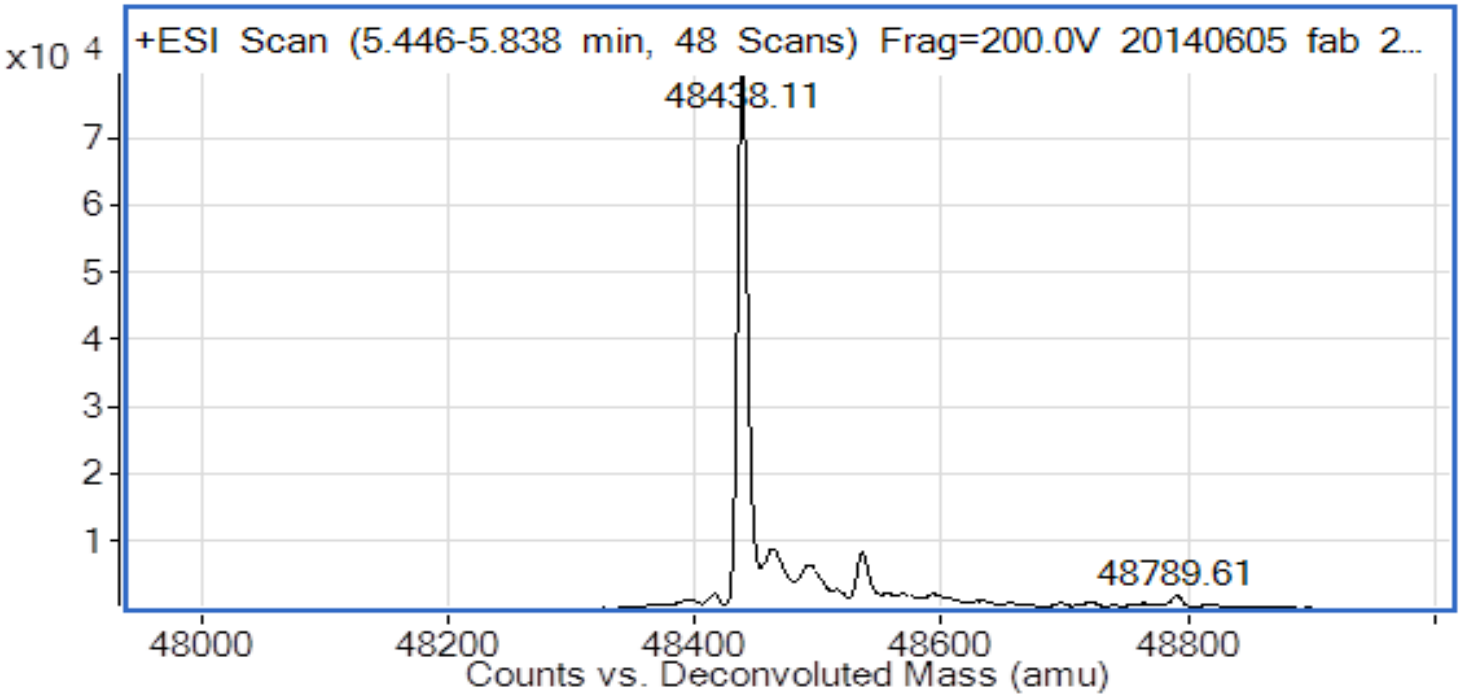


After





Fab Expression with Correct MW



	Theoretical MW	Experimental MW by LC/MS
L-Chain	24395.01	
H-Chain	24043.08	
Fab	**48438.09**	**48438.11**

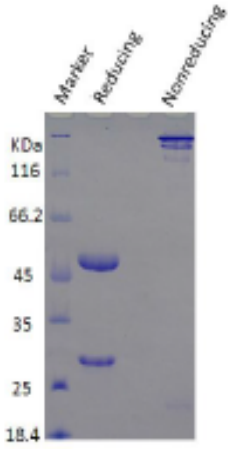
Perfect Match!

COA Examples

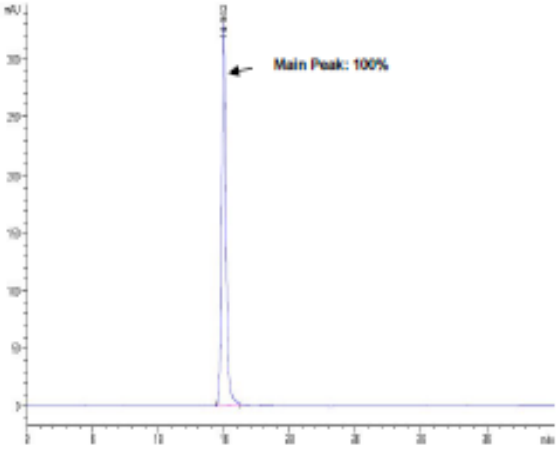
Certificate of Analysis

Date:	Mar 24th, 2014
Name:	MLS128
Lot Number:	20140324
Label on vial:	MLS128, 1.2 mg/vial, Mar 24, 2014
Amount per vial:	Lyophilized 1.2 mg (from PBS pH7.4)
Package content:	1.2mg/vial, 5 vials
Total quantity:	6 mg
Titer:	230mg/L by transient transfection into EXPI293 cells for 5 days
SEC-HPLC:	100%
Purity:	> 95% as estimated by a coomassie blue-stained SDS-PAGE gel
Endotoxin:	0.5 EU/mg
Shipping:	room temperature

12% SDS-PAGE Result



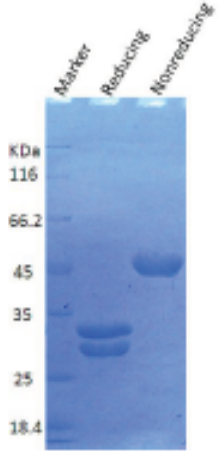
SEC-HPLC (G3000swxl; 0.5 ml/min; 40 min; UV280)



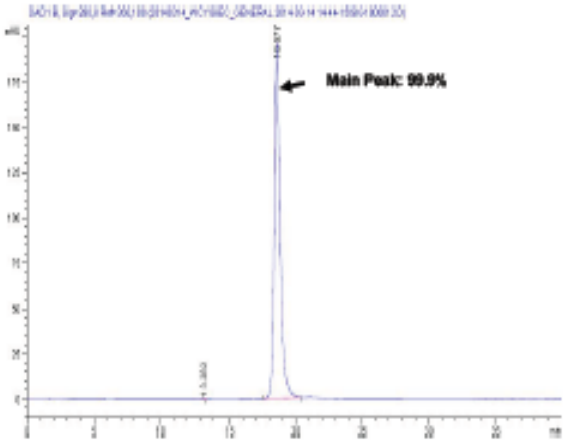
Certificate of Analysis

Date:	Mar 14th, 2014
Name:	Fab 1B4
Lot Number:	20140314
Label on vial:	Fab 1B4, 2.6 mg/ml, 0.5 ml/vial, Mar 14, 2014
Amount per vial:	Lyophilized powder, 1.3 mg in PBS
Package content:	2.6 mg/ml, 0.5 ml/vial, 5 vials
Total quantity:	Lyophilized powder, 6.5 mg
Titer:	90 mg/L by transient transfection into EXPI293 cells for 5 days
SEC-HPLC:	99.9%
Purity:	> 99% as estimated by a coomassie blue-stained SDS-PAGE gel
Endotoxin:	0.5 EU/mg
Shipping:	Dry ice

12% SDS-PAGE Result



SEC-HPLC (G3000swxl; 0.5 ml/min; 40 min; UV280)



Additional Case Study Data

1. Human IgG4 antibody expression

- Started on Feb 10th, 2014
- Completed on Mar 14th, 2014
- Titers ranges from 20 mg/L to 140 mg/L
- Purity >97% by SEC-HPLC, Endotoxin < 1 EU/mg

2. Human IgG1 antibody expression

- Started on Feb 18th, 2014
- Completed on Mar 24th, 2014
- Titers ranges from 190 mg/L to 640 mg/L
- Purity >98% by SEC-HPLC, Endotoxin < 1 EU/mg