QC Biochemistry: Quantification of Anti IL-8 Monoclonal Antibody by ELISA

Purpose:
To quantify the concentration of anti IL-8 monoclonal antibody during the upstream and downstream process of anti IL-8 mAb production**.**

Materials:

- samples from spinner flask day 0 through day 6, bioreactor day 0 through day 5 and from the downstream protein A chromatography
- Materials listed in the material section of "SOP: Quantification of CHO-DP12 derived humanized mouse anti-human IL-8 Monoclonal Antibody by ELISA"

SOP's used:

- SOP: Quantification of CHO-DP12 Derived Humanized Mouse Anti-Human IL-8 Monoclonal Antibody by ELISA
- SOP: Operation of Biorad iMark Microplate Absorbance Reader

These SOP's and other resources can be downloaded from the NBC2 website at
http://biomanufacturing.org/curriculum-resources/program-units/quality-control-biochemistry

Standard curve data for the ELISA:
Anti-IL8 mAb standards from 0 ng/ml to 120 ng/ml were used in the ELISA and the measured absorbances listed Table 1

Table 1

Std conc ng/ml	Abs of std. (1 of 2)	Abs of std. (2 of 2)	Avg. abs.	Abs. - blank
0	0.049	0.033	0.0410	0.0000
10	0.199	0.176	0.1875	0.1465
20	0.342	0.335	0.3385	0.2975
40	0.544	0.496	0.5200	0.4790
60	0.749	0.702	0.7255	0.6845
80	0.936	0.922	0.9290	0.8880
100	0.936	1.000	0.9680	0.9270
120	1.033	1.084	1.0585	1.0175

Quantification of Anti IL-8 Monoclonal Antibody by ELISA Data

Standard Curve:

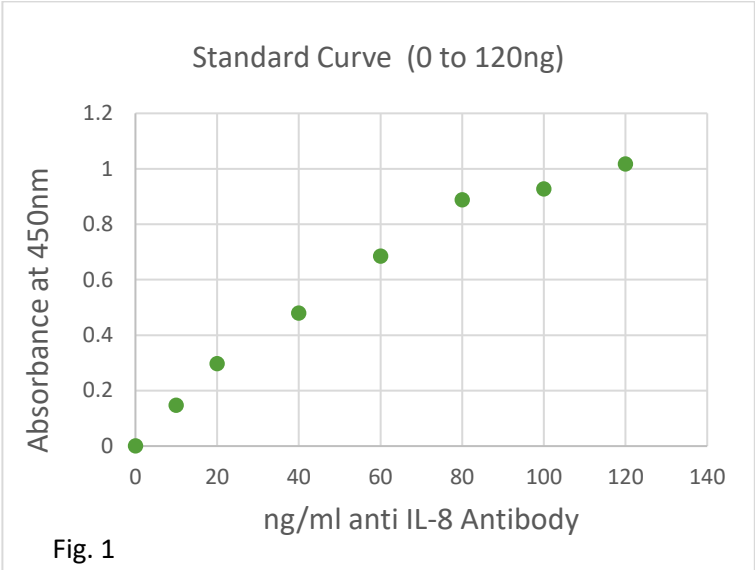


Fig. 1

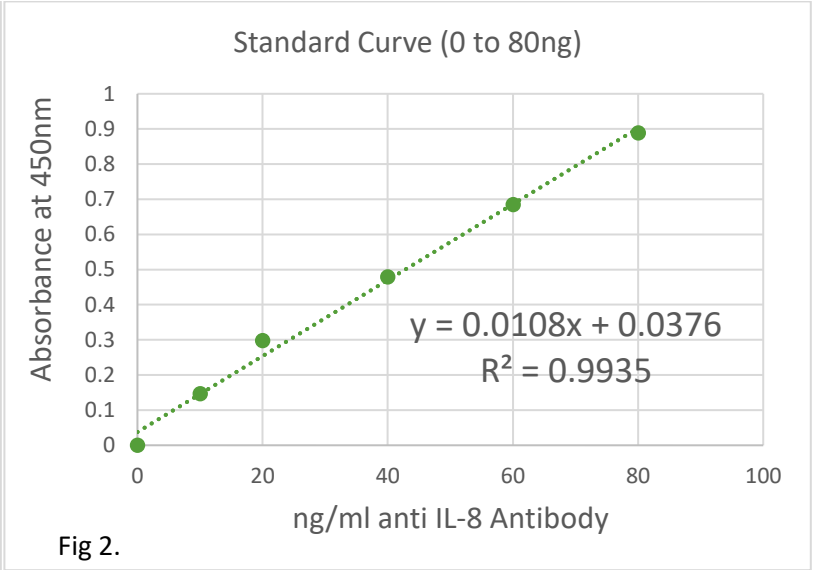


Fig 2.

- Figure 1: Standard curve for concentrations of 0 to 120 ng/ml anti IL-8 antibody
- Figure 2: Standard curve for the linear range of 0 to 80 ng/ml anti IL-8 antibody

Upstream Processing Data:

100 ml of media was inoculated with 2 x 10^7 cells in a spinner flask. The students sampled their spinner flask daily from day 0 to day 6 of the culture. The collected samples were assayed for anti IL-8 mAb concentration to determine the titer of the culture media throughout the growth of the culture.
The average absorbance obtained for the 0ng/ml standard solution, (0.041), was used as the blank value and subtracted from each of the test sample absorbance values.
The equation "y= 0.0108x + 0.0376", of the linear trend line obtained from the std. curve (Fig.2.) was used to calculate the concentration of the spinner flask samples. (table 2)

Spinner flask samples day 0 to day 6:

Table 2

Days	cell concentration cells/ml	Dilutions for ELISA	Abs	Abs-Blank	Anti IL-8 conc ng/ml	Anti IL-8 conc corrected for dilution µg/ml
0	1.50×10^5	0	0.5646	0.5236	45	0.05
1	1.90×10^5	0	0.9426	0.9016	80	0.08
2	3.35×10^5	10	0.4912	0.4502	38.2	0.38
3	4.90×10^5	1000	0.1866	0.1456	10	10.00
4	8.00×10^5	2000	0.4026	0.3616	30	60.00
5	10.00×10^5	2000	0.5106	0.4696	40	80.00
6	11.00×10^5	2000	0.5646	0.5236	45	90.00

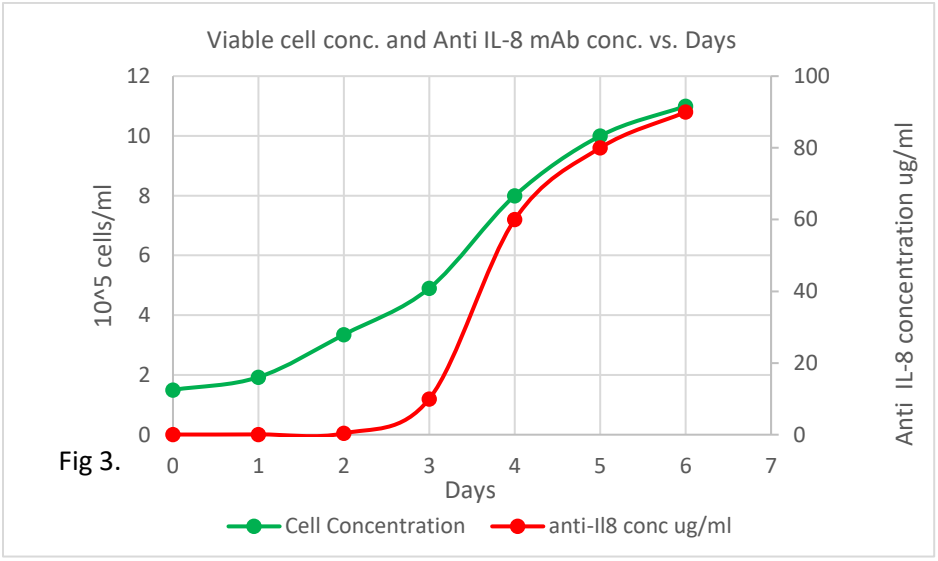


Fig 3.

Figure 3: CHO DP12 growth curve with anti Il-8 mAb titer

From day 0 to day 2 the anti IL-8 mAb concentration is very low as the cells are in lag phase and are not producing antibody. The anti IL-8 mAb concentration increases from day 3 onwards as the cells enter exponential phase of the growth curve and reach a concentration of 90 µg/ml on day 6.

Bioreactor samples Day 0 through Day 5:

The Bioreactor with 1100 ml of media was inoculated with 82 ml of the cell suspension from the spinner flask. Students tested bioreactor samples daily for the anti IL-8 concentration and other critical process parameters starting from day 0 to day 5. The bioreactor was harvested on Day 5.

Table 3

Days	cell concentration cells/ml	Dilutions for ELISA	Abs.	Abs-Blank	Anti IL-8 conc. ng/ml	Anti IL-8 conc, corrected for dilution factor µg/ml
0	0.85×10^5	0	0.9426	0.9016	80	0.08
1	1.19×10^5	10	0.4026	0.3616	30	0.3
2	2.85×10^5	500	0.2514	0.2104	16	8
3	4.65×10^5	2000	0.4026	0.3616	30	60
4	7.60×10^5	2000	0.6186	0.5776	50	100
5	10.40×10^5	2000	0.7914	0.7504	66	132

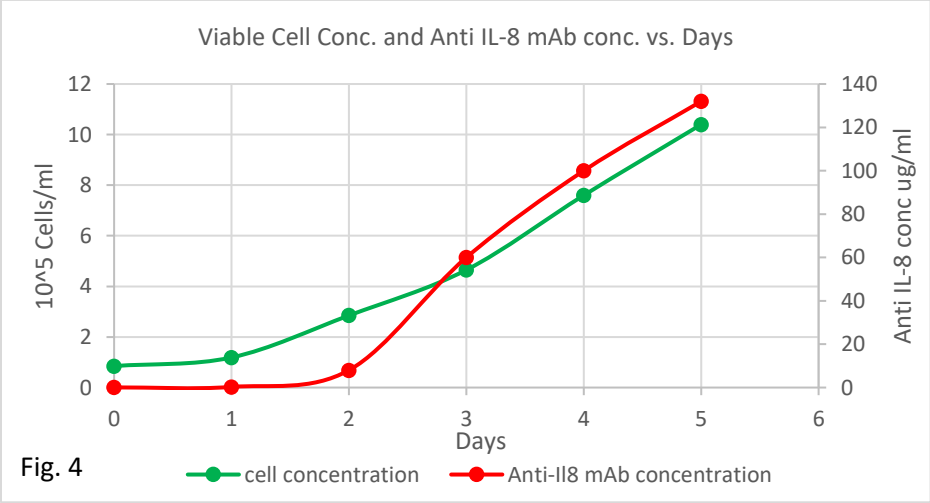


Fig. 4

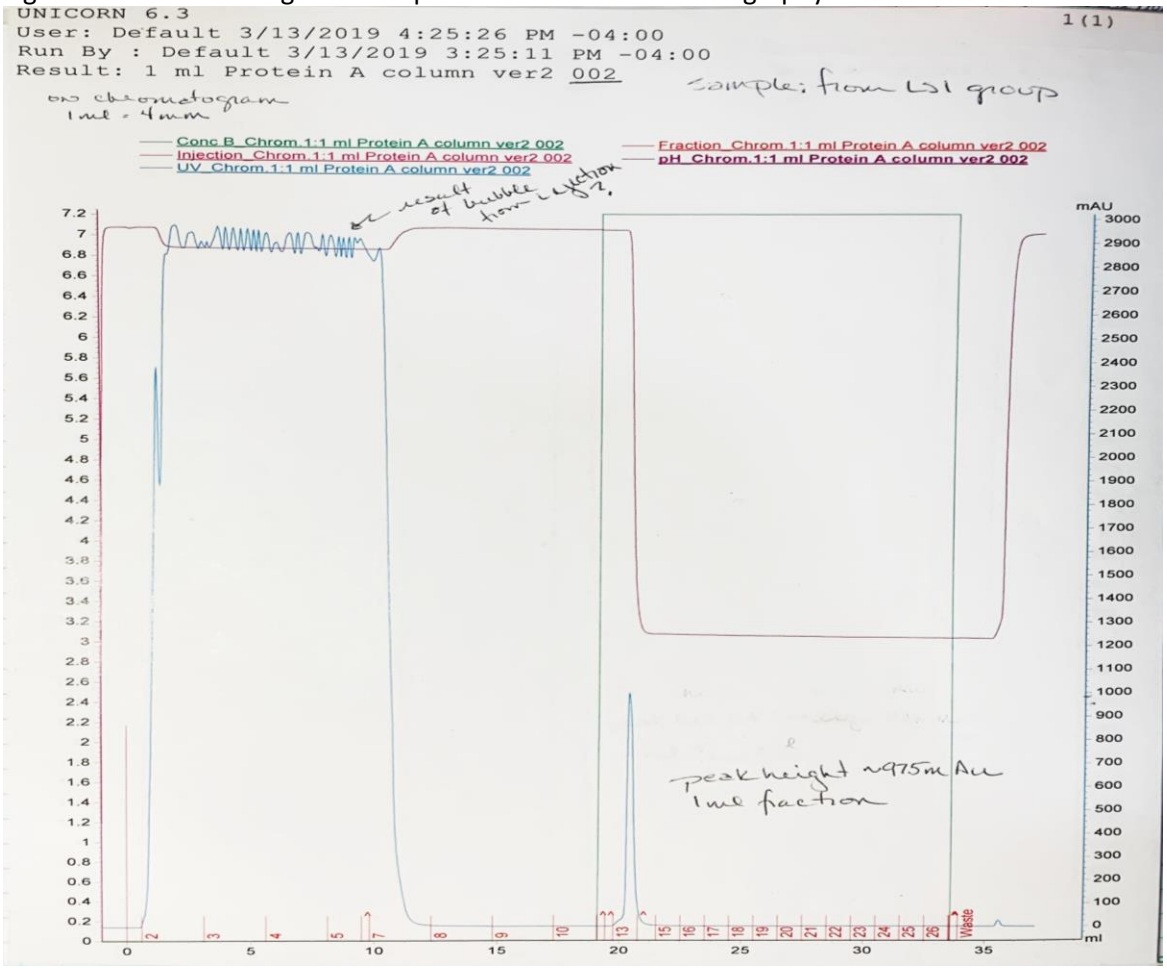
Figure 4: CHO DP12 growth curve with anti IL-8 mAb titer

The concentration of anti IL-8 mAb for day 0 to day 2 is low because the cells are in lag phase. The anti IL-8 mAb concentration increases from day 3 onwards as the cells enter the exponential phase of the growth curve reaching concentration of 132µg/ml.

Downstream Processing Data:

After harvesting the conditioned media from the bioreactor, it was concentrated using tangential flow filtration. A 9.5 ml sample of the concentrated conditioned media was purified using protein A chromatography on an AKTA pure system. The 1.0 ml fractions for the eluted were collected in collection tubes containing 200µl of 1M Tris HCl pH 9.0 to neutralize the low pH of the elution buffer. The peak fraction # 13, contains the purified anti IL-8 mAb. A pre-column sample, a Flow Through sample and the peak fraction 13 were analyzed for anti IL-8 mAb concentration using the ELISA assay

Figure 5: The chromatogram from protein A column chromatography:



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Protein A Purification data:

The Flow Through sample was assayed without further dilution. The peak fraction 13 was assayed at a dilution of 1:20,000. The pre-column load sample was assayed using a 1:2000 dilution.

Table 4

Samples	Abs.	Abs.- blank	Anti IL-8 conc. ng/ml	Actual Anti IL-8 conc. corrected for dilution factor µg/ml
Peak 13 1:20,000	0.5711	0.5301	45.60	912
Flow through	0.0786	0.0376	0	0
Column Load 1:2000	0.6078	0.5668	49	98

- The absence of mAb in the flow through indicates that all the mAb in the loaded material was bound to protein A column
- 9.5ml of TFF concentrated conditioned media results in 912 µg/ml X 1.2ml fraction = 1.094mg of purified mAb of protein A chromatography purified mAb. (The final volume of the peak fraction is 1.2 ml, 1ml of collected sample volume plus the 0.2 ml of TRIS in the collection tube to adjust the final pH.)

Experiments performed and recorded by Dr. Maggie Bryans, Hetal Doshi and Robin Zuck at Montgomery County Community College. Questions regarding data can be sent to mbryans@mc3.edu. This work was funded by NSF ATE DUE 1501631, the Northeast Biomanufacturing Center and Collaborative.