

## INTRODUCTION

Monoclonal antibodies from hybridoma cell supernatant are used for a variety of medical and research purposes but must be purified before use. Monoclonal antibodies obtained from hybridoma cell supernatant were purified using a protein A/G agarose resin bead affinity chromatography column. Antibody identity was determined using SDS-PAGE. Antibodies recovered from affinity column were quantified using two industry standard methods: UV-Visible Spectroscopy and the Bradford Assay. Quantitative results are discussed.

## METHODS

Monoclonal antibodies (IgG) were recovered from 25 mL of hybridoma cell supernatant using protein A/G affinity chromatography (Figure 1). Antibody was washed from column with pH 2.5 buffer. Physiologic pH was reestablished using pH 9 neutralization buffer. Dialysis was performed on pure product to remove excess ions.

- SDS-PAGE separates protein by molecular weight and was performed to determine the identity of the protein recovered from column. Antibody should produce bands at 25 kDa and at 50 kDa (Figure 2).

- Bradford assay was used to determine concentration of protein in  $\frac{mg}{mL}$ . The Bradford assay measures the absorbance shift from 465 nm to 595 nm of the Coomassie Brilliant Blue G-250 reagent when bound to protein. A standard curve was generated using bovine serum albumin (BSA) concentration and absorbance at 595 nm (Figure 3).

- Nanodrop 2000c spectrophotometer (Figure 6) absorption data at 280 nm wavelength was used to calculate protein concentration, from spectrum generated (Figure 7), in  $\frac{mg}{mL}$  using the Beer-Lambert law  $A = \epsilon b c$  where  $A$  = absorbance,  $\epsilon$  = molar extinction coefficient,  $b$  = path length (1mm),  $c$  = concentration. In this case  $\epsilon$  of IgG is assumed to be  $13.7 \frac{L}{g \cdot cm}$ .

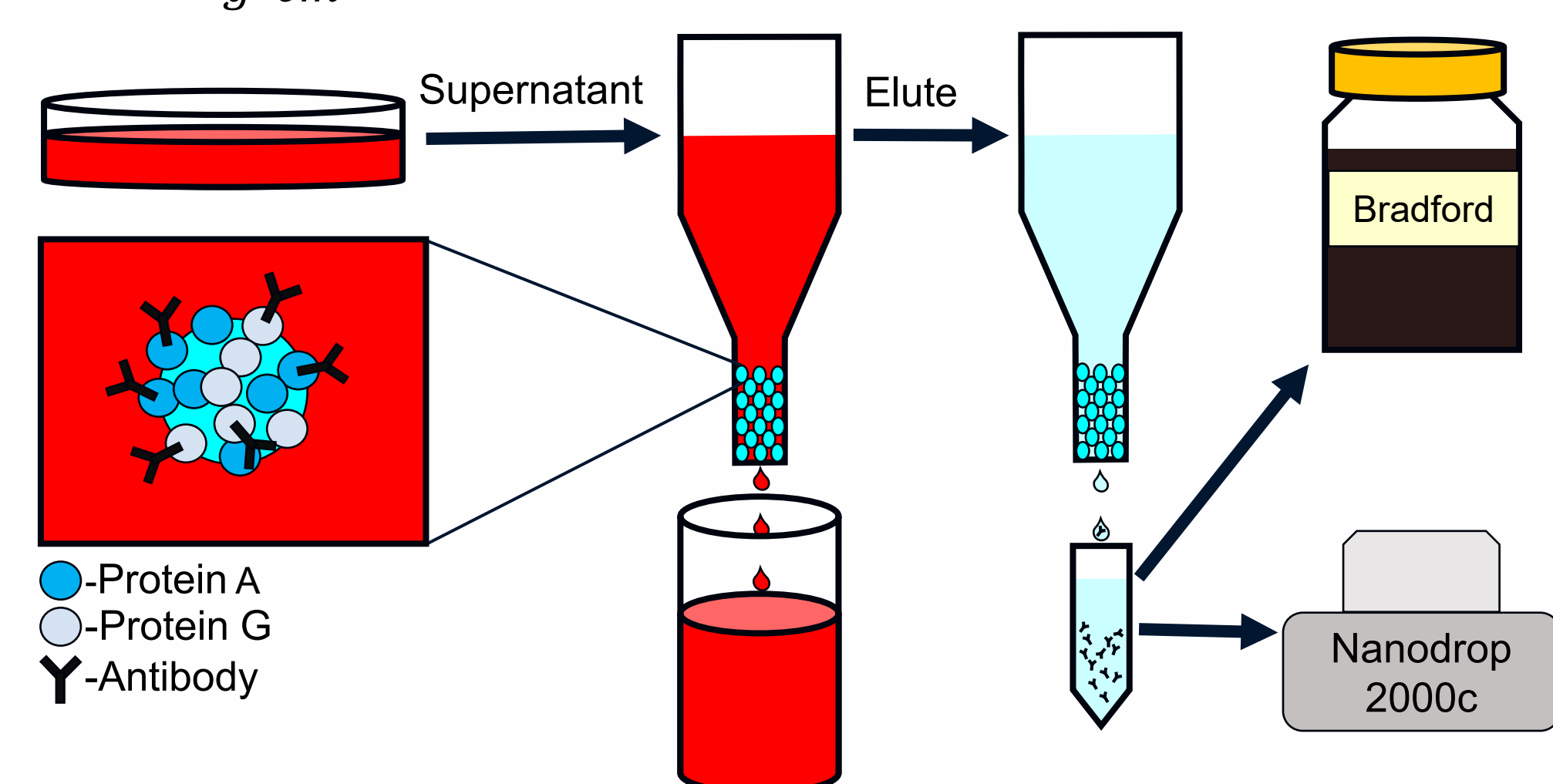


Figure 1. workflow of protein A/G affinity chromatography to prepare pure monoclonal antibody for quantification.

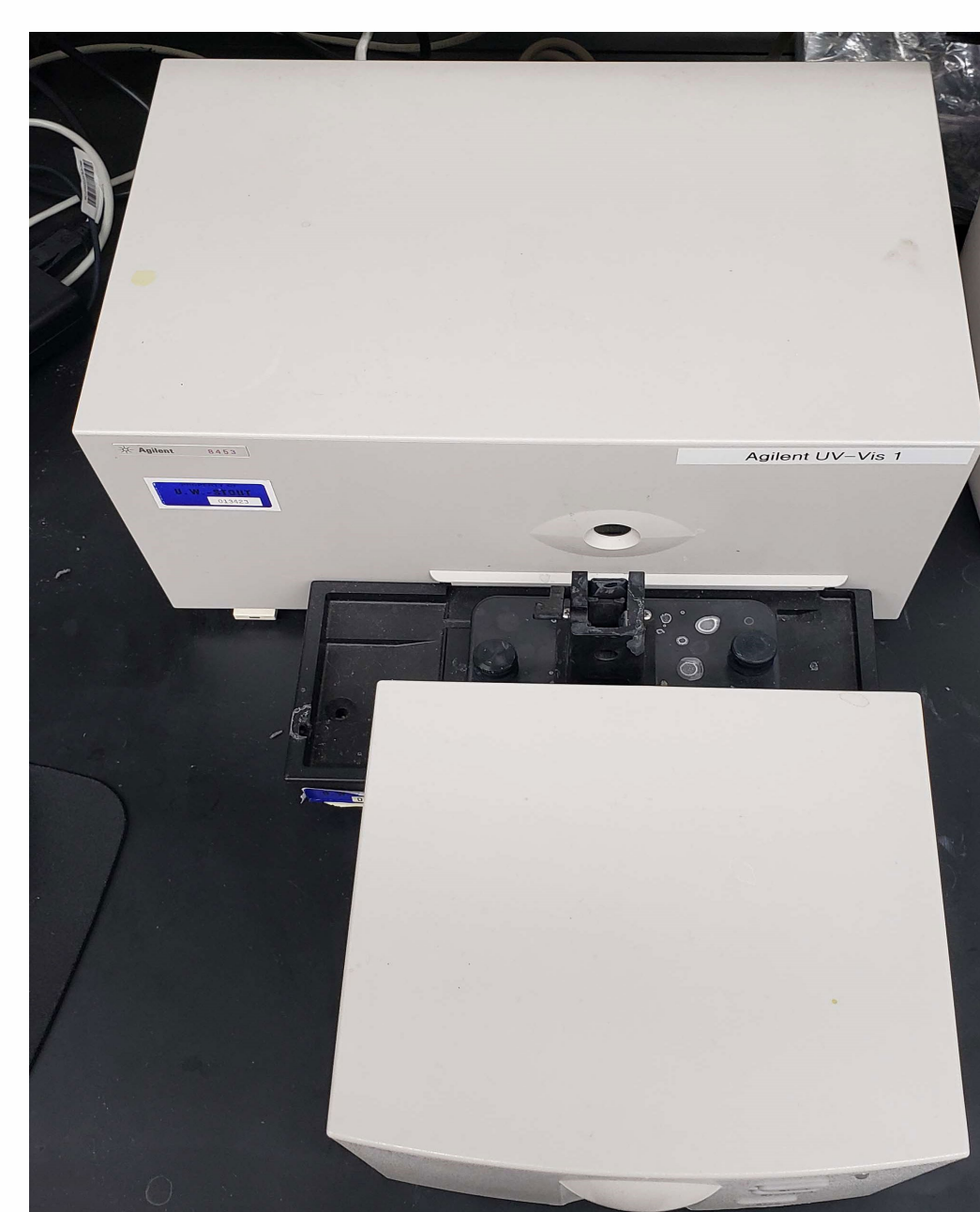


Figure 4. Image to the left is the Agilent 8453 UV-visible spectroscopy system used to generate standard curve of BSA and quantify IgG with Bradford assay.



Figure 6. Image on the right is the Nanodrop 2000c spectrophotometer used to calculate concentration of IgG using 2 µL of sample.

## RESULTS

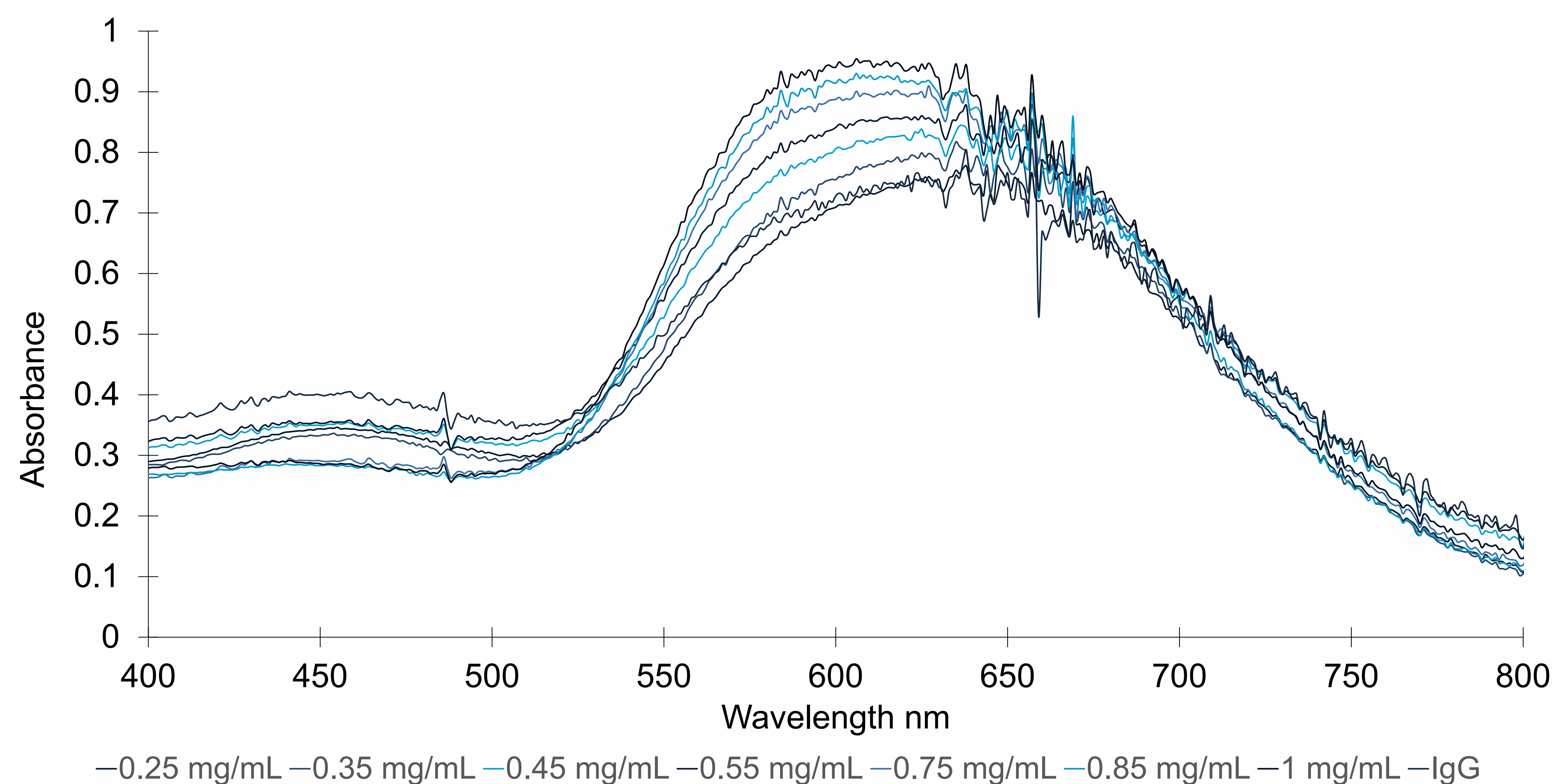


Figure 3. Spectra of wavelength vs absorbance of bovine serum albumin and IgG generated with the Agilent 8453 UV-visible spectroscopy system (Figure 4). Deionized water was used as a blank. These spectra were used to obtain absorbance of bovine serum albumin to generate a standard curve (Figure 5).

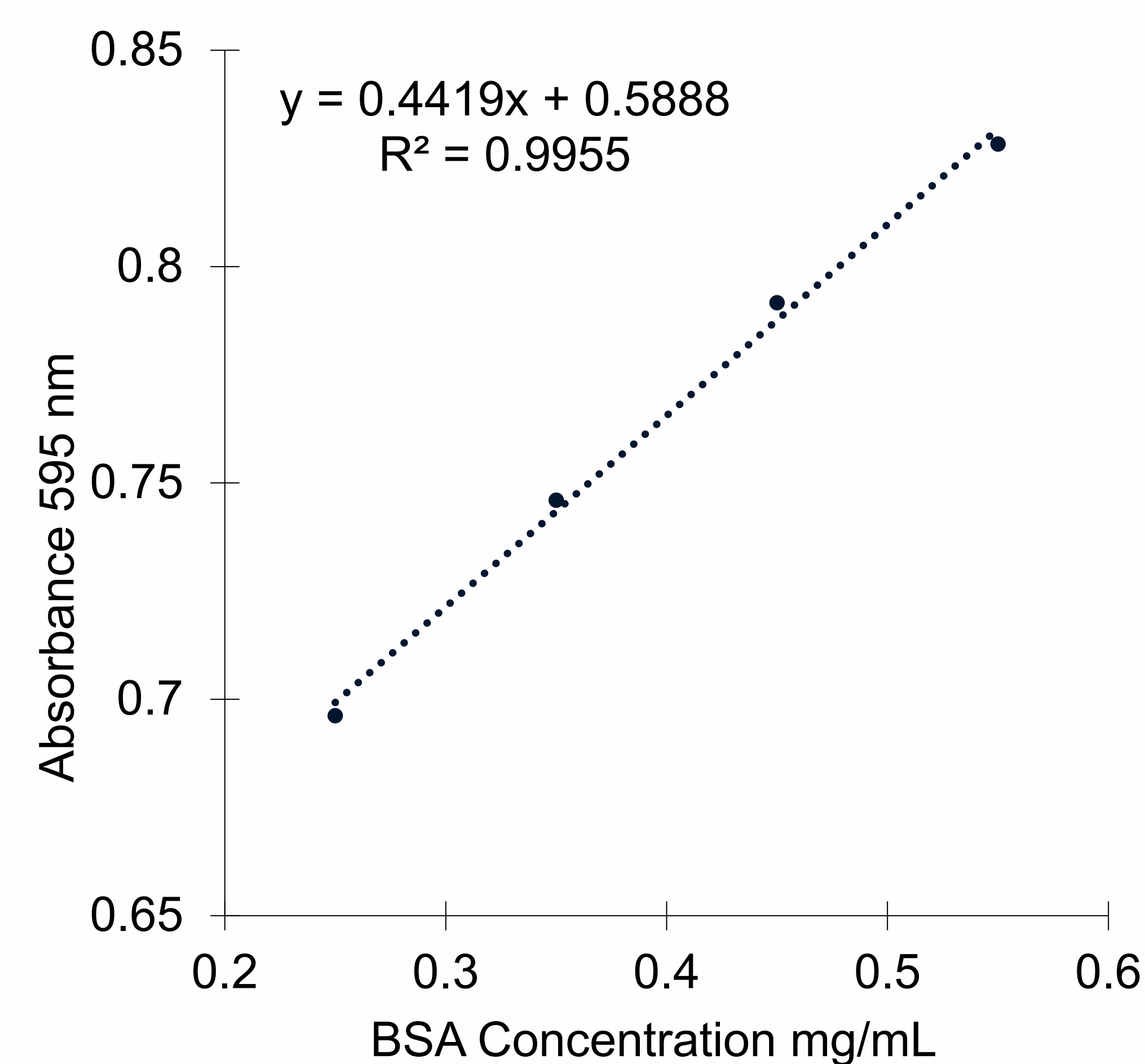


Figure 5. Standard curve of bovine serum albumin (BSA) calculated using the absorbance at 595 nm from the spectra in Figure 4, and the concentration of BSA used. Curve flattens at concentrations above 0.55 mg/mL, these concentrations were omitted.

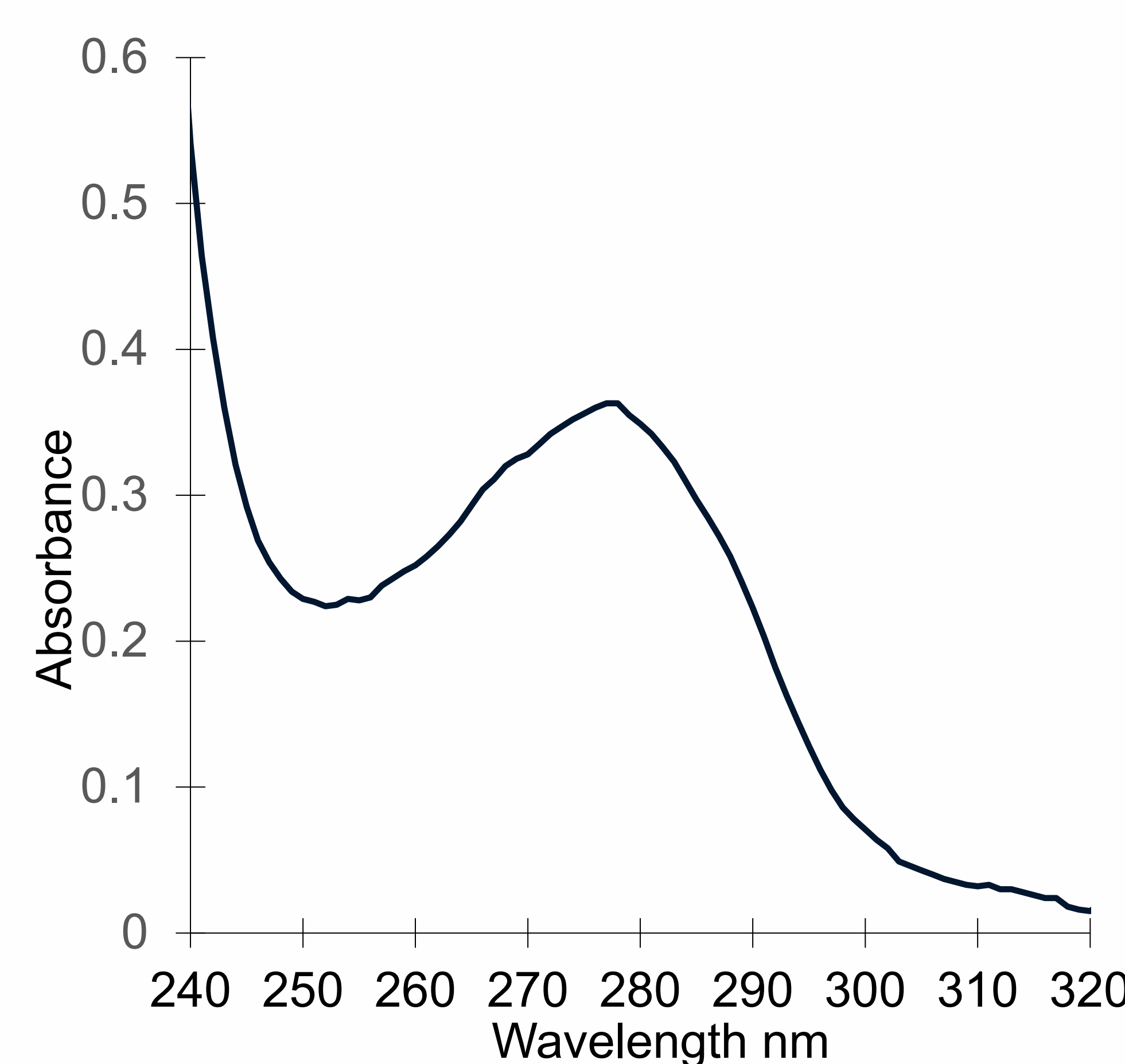


Figure 7. Spectra generated using Nanodrop 2000c spectrophotometer. Concentration of IgG was calculated using the absorbance value of 0.349 at 280 nm wavelength. Nanodrop was blanked with 0.1 M Tris HCl buffer.

Method	IgG concentration (mg/mL)
Bradford Assay absorbance 595 nm	0.263 mg/mL
Absorbance 280 nm with Nanodrop 2000c	0.255 mg/mL

## DISCUSSION

The result of this study showed that Bradford Assay and absorbance at 280 nm produced similar results when determining protein concentration in  $\frac{mg}{mL}$ . The percent difference between the two methods is 3.09%, with a  $\alpha = 0.95$ , this percent error is an acceptable value and both quantification methods are equally useful. Unfortunately, the SDS-PAGE did not conclusively reveal that the protein isolated was IgG. While affinity chromatography is a very specific method for compound isolation without a second confirmation of the identity of the compound, such as SDS-PAGE, it is impossible to say if what was isolated is truly IgG. Both methods of quantification used in this study have the limitation of being able to tell us how much protein is present, but not how many protein molecules are present. Antibodies are composed of two heavy chains and two light chains, each containing a variable region and a constant region. A protein with a variable region such as an antibody makes it quite difficult to determine molar concentration of the protein, even if techniques such as mass spectrometry were used for quantification. For true quantification of an antibody, a genetic sequence of the antibody producing region would be required to determine the correct amino acid proportions.

## CONCLUSIONS & RECOMMENDATIONS

Affinity chromatography is a very specific way to isolate antibody from cell culture supernatant. From absorbance data it is apparent that some protein was removed from the affinity column. Uncertainty of the identity of the recovered protein warrants further investigation. The Bradford Assay is a valid way to determine concentration in  $\frac{mg}{mL}$ , but it is much more labor intensive than obtaining the absorbance of sample at 280 nm. The benefit to the Bradford Assay is the comparison of unknown protein to a standard curve. Performing both methods gives a more accurate measurement of the amount of protein present in the sample. For industrial purposes it would be much more suitable to determine concentration from absorbance at 280 nm due to how rapidly the spectrum can be generated. Unfortunately, neither method of quantification gives a truly quantitative value, just a rough estimate.

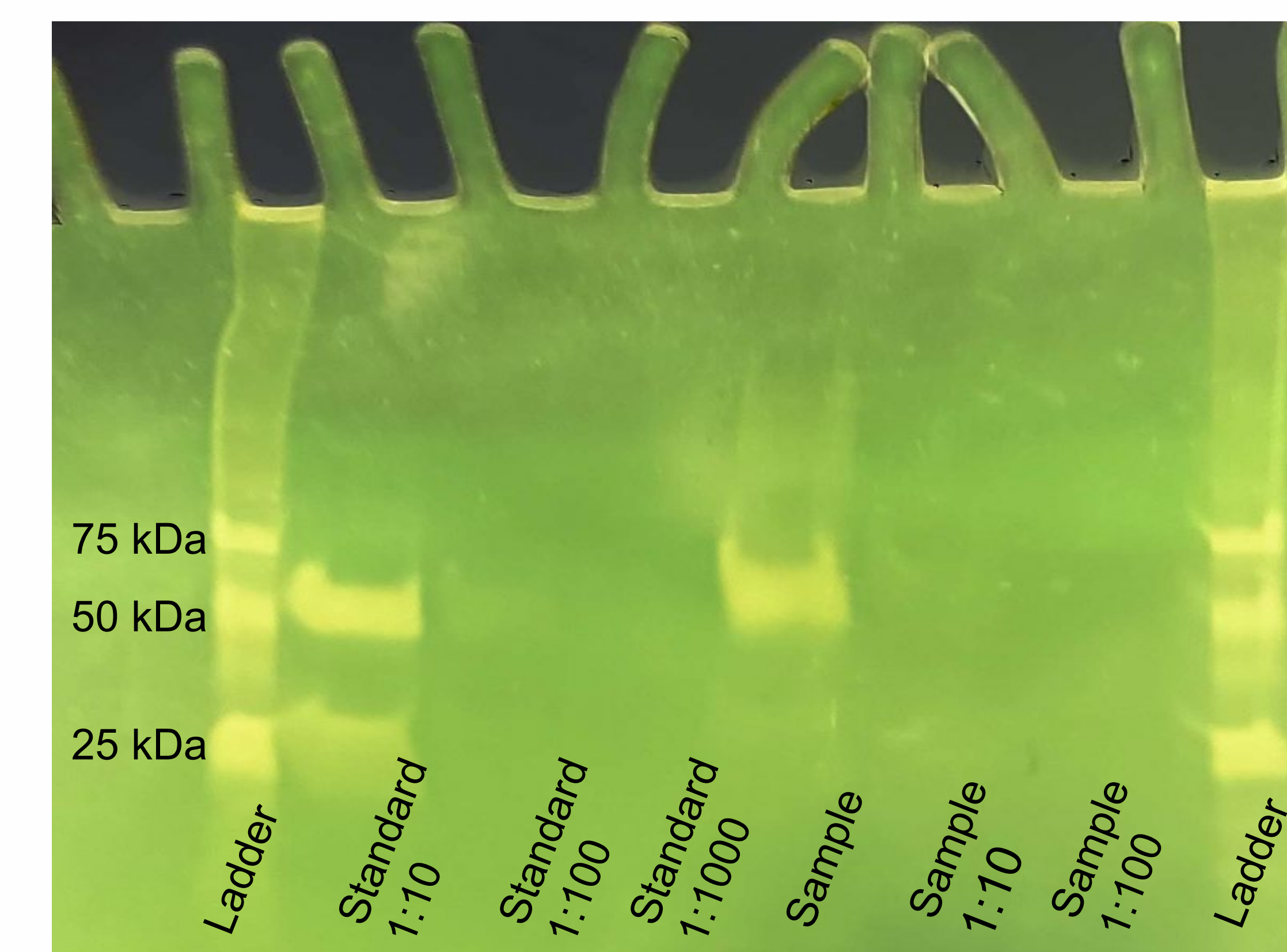


Figure 2. SDS-PAGE gel stained with Coomassie Brilliant Blue R-250. Color is inverted for better visualization. Ladder used was Bio-Rad Precision Plus Protein Unstained Standard catalog #161-0363. Mouse IgG 10.7  $\frac{mg}{mL}$  from Thermo Fisher Scientific was used as a standard. Sample is eluant recovered from column. Serial dilutions of each were performed.

## REFERENCES

- NanoDrop 2000/2000c Spectrophotometer V1.0 User Manual (2009). Thermo Fisher Scientific
- Bradford M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72, 248–254. <https://doi.org/10.1006/abio.1976.9999>
- Pierce Protein A/G Agarose [Instruction Manual](2016) Retrieved from [https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2Fmanuals%2FMAN0015861\\_2160128\\_PierceProtein\\_AG\\_Agarose\\_UG.pdf](https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets%2FMSG%2Fmanuals%2FMAN0015861_2160128_PierceProtein_AG_Agarose_UG.pdf)

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