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## Partial Purification of Alzheimer's Amyloid- $\beta$ Specific Antibody Using Ammonium Sulfate

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# Partial Purification of Alzheimer's Amyloid- $\beta$ Specific Antibody Using Ammonium Sulfate

## Abstract:

- Alzheimer's disease (AD) is the most common neurodegenerative disease. The trigger for AD is the accumulation of amyloid-beta protein ( $A\beta$ ) as senile plaques in the brain. Prior to forming the fiber-like structures found in the plaques,  $A\beta$  undergoes an oligomerization process that produces intermediate structures called protofibrils. Substantial data from the Nichols laboratory demonstrated that soluble  $A\beta$  protofibrils were highly inflammatory compared to other forms of  $A\beta$ . Based on these findings, a serum polyclonal antibody, named Antibody St. Louis or AbSL, was developed to target  $A\beta$  protofibrils. A significant challenge with serum antibodies is the presence of many other biological factors in the samples. This study investigates the application of ammonium sulfate (AS) to precipitate and partially purify the AbSL antibody from serum, aiming to enhance its sensitivity and specificity. Two separate purification experiments indicated that the serum AbSL antibody could be precipitated between 20% and 50% AS. The AbSL antibody was tracked in different fractions using an ELISA assay. Further studies will help determine the exact solution that will give optimal purification of the AbSL serum antibody. This method will effectively eliminate extraneous serum proteins that could potentially impede the functionality of the antibody.

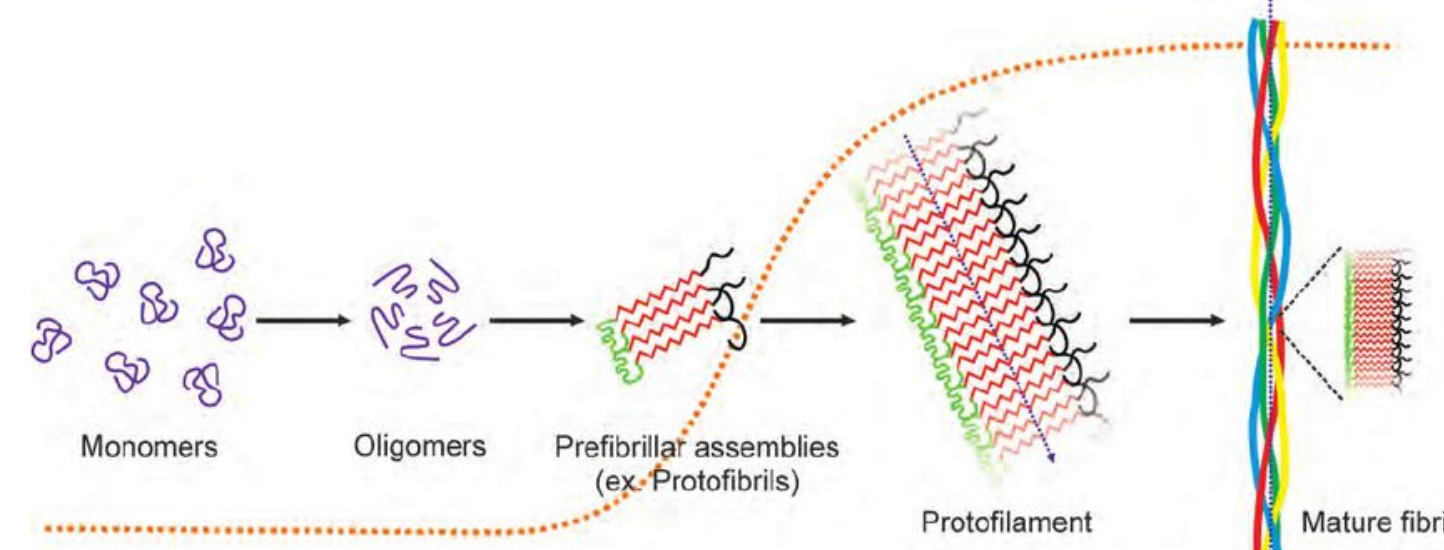


Figure 1  $A\beta$  Oligomerization: Demonstrates the step by step process of  $A\beta$  monomers turning into mature fibrils that are evident in late stage Alzheimer's patients

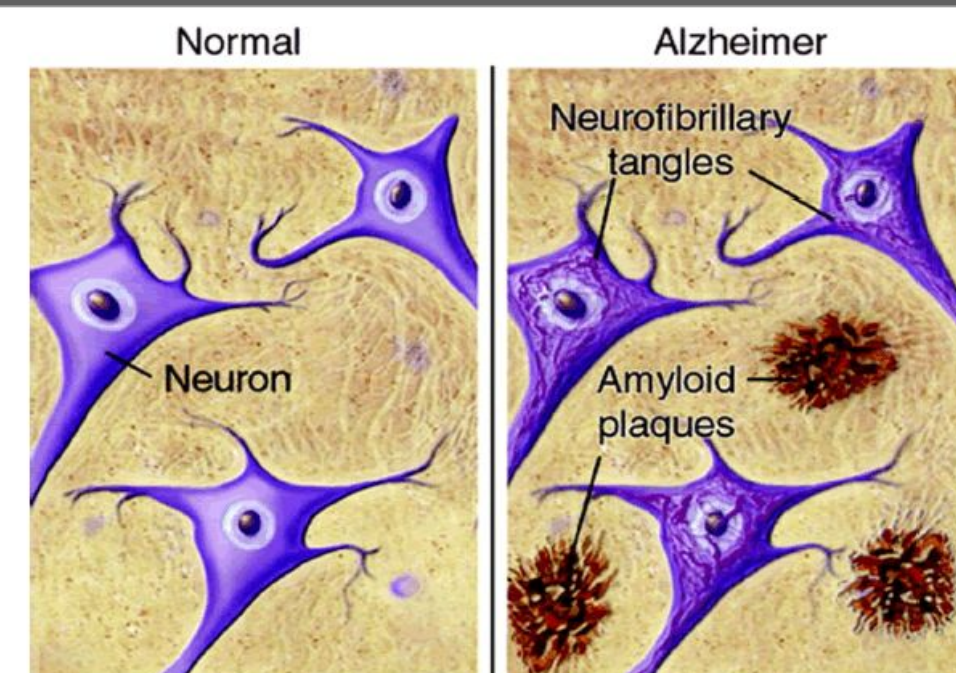


Figure 2 Morphology of AD: Alzheimer's Disease is characterized by extracellular Amyloid plaques (shown in yellow) that form between neurons and disrupt their ability to communicate and send signals to each other.

## Objectives:

- Assess the impact of varying concentrations of AS on the precipitation and purification of AbSL antibody from serum samples 39-1 and 40-4.
- Compare the antibody content in serum samples before and after AS precipitation using UV absorbance and enzyme-linked immunosorbent assay (ELISA).
- Evaluate the feasibility of integrating AS precipitation into the production process of AbSL antibody for enhancing its sensitivity and specificity in targeting  $A\beta$  protofibrils.

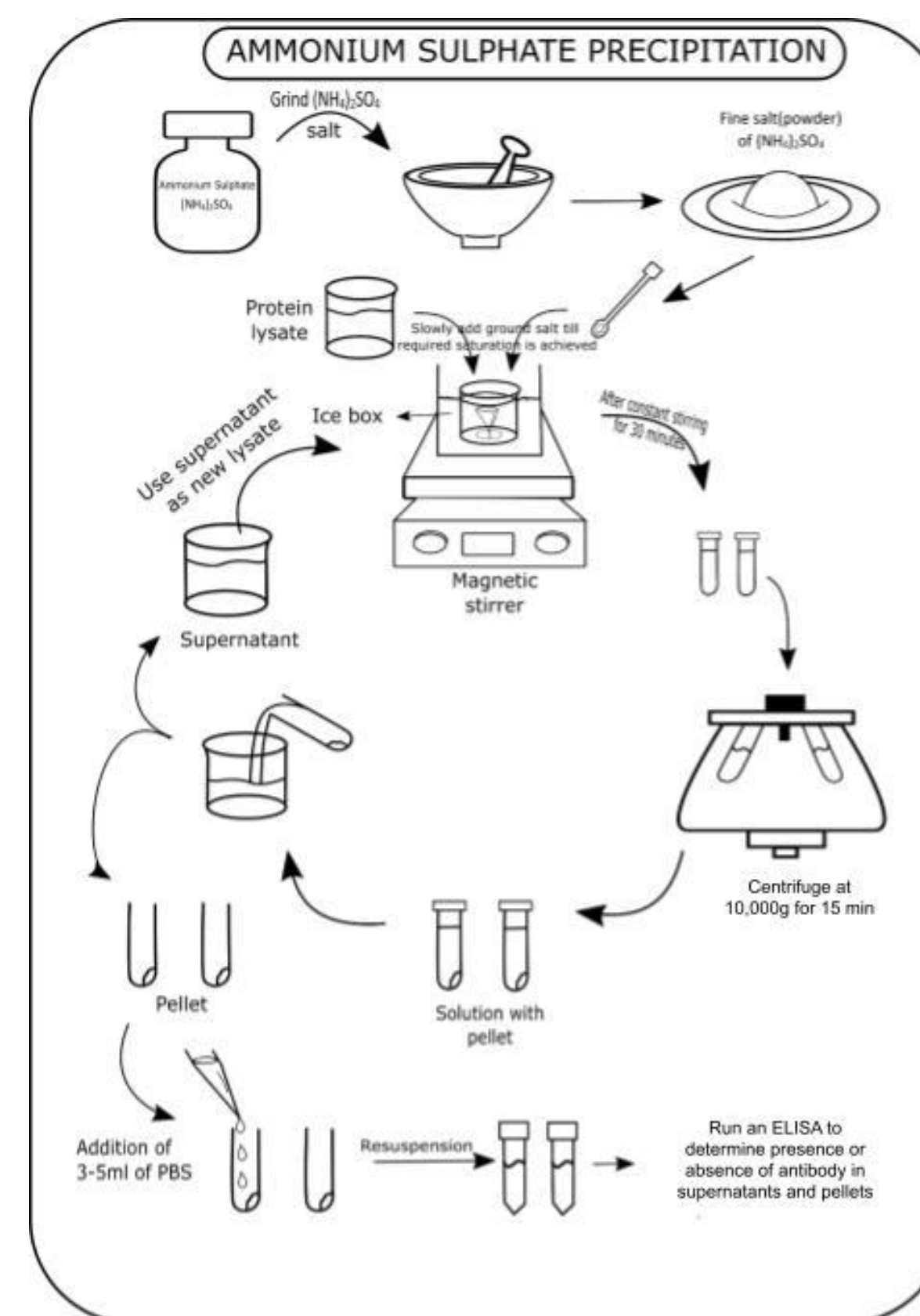
## Methods:

### 1. Ammonium Sulfate (AS) Precipitation

- Samples were centrifuged at the same speed and time to pellet the precipitated proteins; pellet and supernatant were separated into two different microtubules.

### 2. Protein Pellet Resuspension

- Pelleted proteins in both 20% and 50% saturation were reconstituted in 0.4mL of phosphate-buffered saline (PBS).
- Care was taken to ensure complete dissolution of the pellet through gentle agitation or mixing



### 3. Enzyme-Linked Immunosorbent Assay (ELISA)

- The presence of AbSL antibody in each sample was assessed using ELISA.
- Microtiter plates were coated with  $A\beta$  protofibrils as the capture antigen.
- Samples were added to the wells and incubated to allow binding of the antibody.
- Detection was performed using secondary antibodies conjugated with horseradish peroxidase (HRP) and a colorimetric substrate.
- Antibody concentration in each sample was determined using UV absorbance at 280 nm.

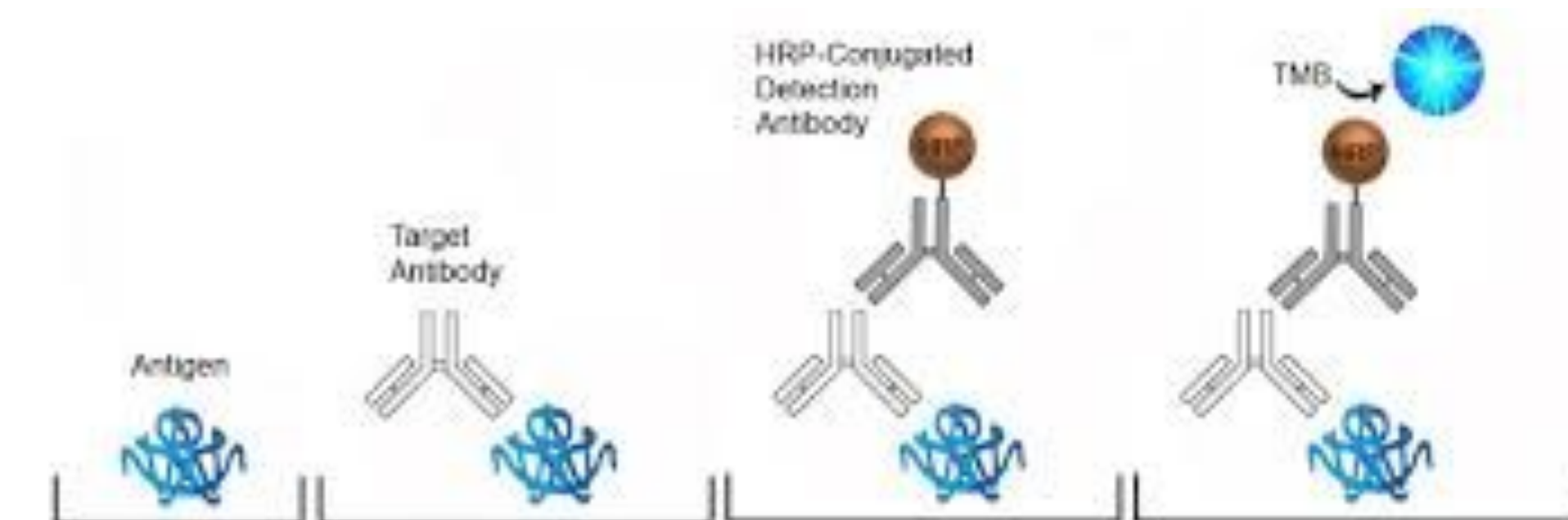


Figure 3 Indirect ELISA Diagram: The plate is coated with a capture antibody. The sample is added, and any antigen present binds to capture antibody. The detecting antibody is added, and binds to antigen. HRP is added as well as TMB. This allows us to quantify the specificity of such antibody for the different  $A\beta$  intermediates.

## Conclusion:

- The antibody seems to be present in both the initial centrifugation of the source serum as well as in the low 20% saturation level pellet
- The application of AS precipitation demonstrated an increase in sensitivity and specificity of the AbSL antibody, as evidenced by UV absorbance and ELISA analysis.
- Future research could further optimize the AS precipitation protocol to maximize the yield and purity of the AbSL antibody while minimizing non-specific binding and contamination.

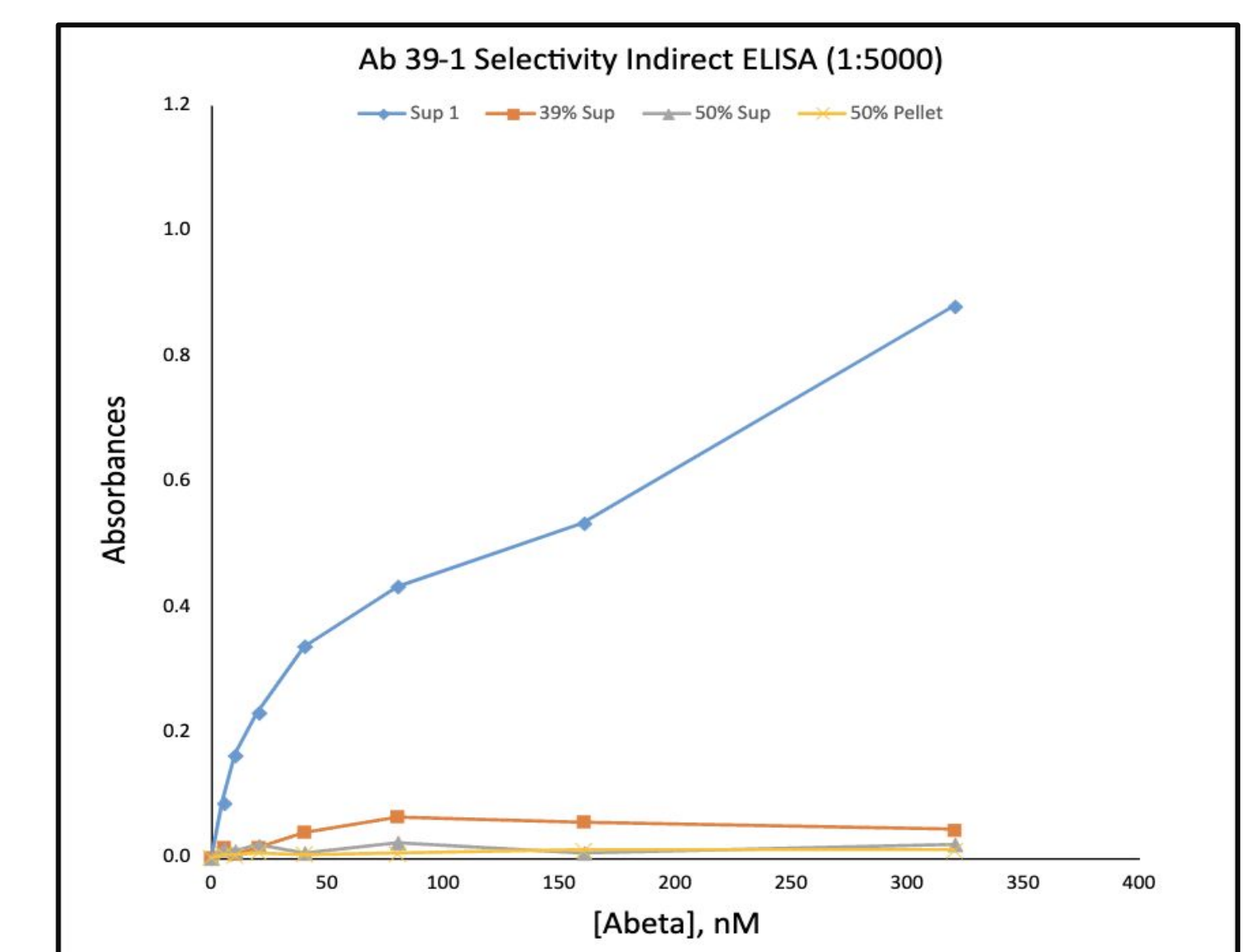


Figure 4 Data Analysis of first trail: The first centrifuged sample of 39-1 showed the highest amount of antibodies as seen by the increased absorbance. The data and spreadsheets are not yet completed or final products of the experiment due to inconclusive results. Polishing on the graphs and data needs to be further analyzed to ensure more during future experiments.

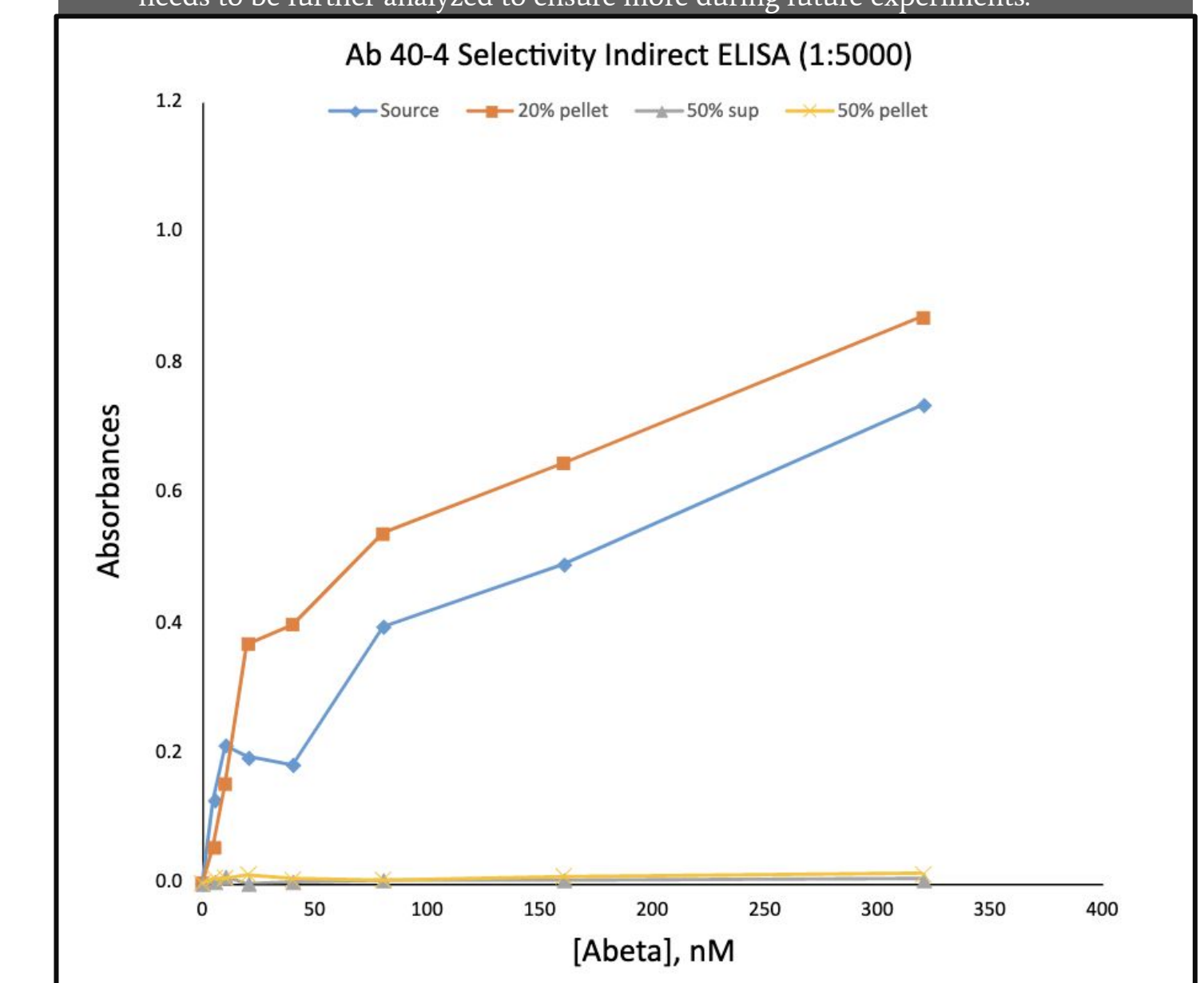


Figure 5 Data Analysis of second trail: As seen from the previous graph with Sup 1, the source has quite a bit of antibody in its presence. The PBS dissolved pellet after saturating it 20% of the way with AS shows an increase in antibody specificity. Results for why this data occurs is not yet complete, and further research is needed to conclude the reason behind this.

## References:

- Salazar, A. (2017). *Indirect ELISA*. Creative Biolabs. Retrieved April 6, 2024, from <https://www.google.com/url>
- National Institute on Aging. (2019). *Alzheimer's Disease*. Alzheimer's Los Angeles. National Institute on Aging. Retrieved April 6, 2024, from <https://www.alzheimersla.org/for-families/understanding-memory-loss/alzheimers-disease-the-basics/>.