Experiment StandardCurve\_001JF

## Title: Using a standard curve to quantify unknowns

Principal Investigator(s)**:**

Protocol prepared by **JF 18 January 2011**

Modified by **JF 30 August 2017**

## Objectives

The purpose of this study is to:

1. Practice calculating dilutions and making solutions
2. Develop and learn how to use a standard curve to quantify concentrations of some unknown component (e.g., protein) in a sample

**Procedure**

**Prepare BGG standard curve using saline.**

1. Prepare dilutions of a protein standard containing from 0.2 mg/ml to about 1.5 mg/ml protein following the table below. A standard curve should be prepared each time the assay is performed. *For best results, the standard should be prepared in* ***the same buffer*** *as the sample.*
2. **Calculate the amount of stock and saline** **needed to make 100 uL** of each target BGG diluted standard. You will need to know how to do this for an exam!!!!

Table 1. Prepare 100µl of each dilution using 2.0 mg/ml (=2000ug/ml) BGG stock:

|  |  |  |
| --- | --- | --- |
| Target BGG Conc. (ug/ml) | 2 mg/ml BGG stock (ul) | Saline (ul) |
| 0 |  |  |
| 200 |  |  |
| 400 |  |  |
| 600 |  |  |
| 800 | 40 | 60 |
| 1000 |  |  |
| 1200 |  |  |
| 1500 |  |  |

**Be sure to use the same units for Cs (s=stock) and Cd (d=diluted) when using the equation”**

**Use CsVs = CdVd** to calculate volumes above.

c. Once you have had your dilution calculation checked by the instructor, proceed to step #2.

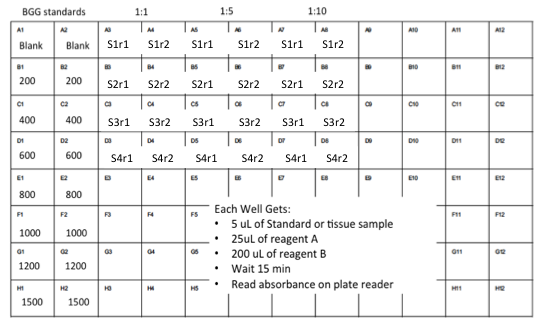
1. Obtain your unknown protein sample from the instructor.
2. The sample must be diluted three different ways to test which dilution is within the range of the protein (BGG) standard curve. You will need all three dilutions for your unknown sample, so each person in your group will end up with a total of 3 labeled microtubes. Calculate the amount of unknown protein sample and saline needed **to make 50 uL of each dilution**.

|  |  |  |
| --- | --- | --- |
| Dilution | Unknown protein sample (uL) | Saline (uL) |
| 1:1 | **50** | 0 |
| 1:5 |  |  |
| 1:10 |  |  |

1. Ensure that all dilutions are thoroughly mixed by either vortexing or palpation. The diluted protein sample should be kept on ice and are now ready for downstream assays.

**Quantify total protein in unknown sample.**

1. Design your 96-well plate as follows for BGG standard curve and for the 1:1, 1:5, and 1:10 factorial dilutions of your unknown protein sample for each person in your group (S# = student #, r= replicate # – if you have 5 people in your group, you will add an additional set of dilutions in row E).



**You must have standards and samples in the EXACT location as described on this plate!!!! Use a grease pencil to remind you where things go, but DO NOT write on the bottom of the plate (Why is this important?).**

Once you are sure which standards and samples will go where, proceed to step 3

**THE ORDER OF REAGENTS MATTERS, FOLLOW THE NEXT STEPS EXACTLY!!**

1. Pipet 5 µl duplicates of each standard or diluted unknown sample into the microtiter plate according to your diagram for the 96-well plate.
   1. Fresh protein standards will go in columns 1 and 2.
   2. Replicates of 1:1 dilution samples for Student #1 will go in the first row (A) of column 3 and 4.
   3. Repeat this pattern for the 1:5 and 1:10 diluted samples for each student in your group in appropriate columns and rows as shown in 96-well plate above.
2. After ALL dilutions of unknown protein samples from each person in your group are in wells, add 25 µl of reagent A into each well.
3. Add 200 µl reagent B into each well. Gently tap the edges of the plate to mix the reagents. If bubbles form, pop them with a **clean**, dry pipet tip. Be careful to avoid cross-contamination of sample wells.
4. After 15 minutes at room temperature, read absorbances at 750 nm. The absorbances will be stable for about 1 hour.

**Expected results:**

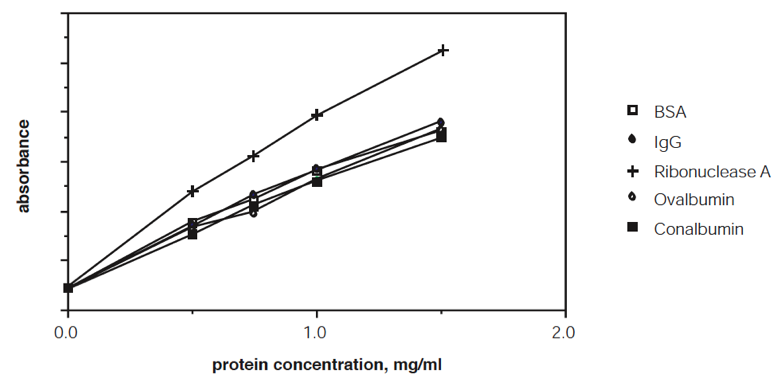


Fig 1. Representative relationship between protein concentration in standards and measured absorbance. As with any colorimetric assay, different proteins will elicit greater or lesser color formation. The following proteins have been assayed with the protein assay. As demonstrated by the graph, there is a slight variation in color development with different proteins.

**Enter results in google docs and complete analysis**

1. Determine relationship between protein concentration and absorbance for your BGG standards.
2. Use linear standard curve equation to calculate concentration of protein for each of your dilutions of your unknown sample. Are there any of your dilutions where you should not use the standard curve to determine protein concentration (i.e., below or above the curve)? Why is this important to consider? How does this influence interpretation of your results?
3. Correct for the dilution factor to determine protein concentration in your actual sample.
4. Enter dilution corrected concentration for each of the dilution in the class data spreadsheet.
5. Statistically compare the dilution corrected concentrations of your three diluted unknown samples and prepare a short report of results.

**Potential questions to consider for an exam:**

1. What is the range of protein concentrations in samples that you can accurately quantify?
2. What would be a better protein than BGG to use if you were measuring a specific enzyme of interest?
3. What processes during this experiment could decrease the concentration of protein in the samples?
4. What would you do if your absorbance reading of a sample is higher than the absorbance reading of your 1500 ug/mL standard of BGG?