

Biochemistry Discussion

BI/CH 421/527/621

Fall 2014

Jose Medrano & Quinn Ho

Discussion sections

C1: M 10-11, CAS B36

C2: M 3-4, CAS 326

C3: T 8-9, CAS B36

C4: T 12-1, PSY B43

Office hours and contact info

W 11:30-12:30, LSE 704

(and by appointment)

biochemlabcoordinators@gmail.com

Laboratory sections

B1: T 4-8

B2: W 8-12

B3: W 1-5

B4: W 6-10

B5: R 8-12

B6: R 1-5

B7: R 6-10

B8: F 8-12

B9: F 1-5

BC: F 6-10

BA: M 11-3

BB: M 4-8

**All lab sections are held in
SCI162 unless stated otherwise**

Biochemistry Discussion

Discussion will provide helpful notes to complete lab experiments and **notebook write-ups** for each chapter.

Quizzes will be given at the end of every discussion (take-home quiz for today only).

A lab compartment grade is assigned mid-way through the semester (not counted) and at the end of the semester (**final compartment grade**).

Lab exam held during the last week of discussion (Dec 8 & 9). Comprehensive written exam testing knowledge of lab concepts & procedures.

Lab grade is composed of:

Lab grade component	Percentage
Notebook write-ups	70%
Discussion quizzes	10%
Final lab compartment	10%
Lab exam	10%

Lab syllabus

Prerequisites

- Must have earned a “C” or higher in Organic Chemistry II

Questions, concerns, differences in opinion

- Resolve it with your lab TF first
- First TF is unavailable? Try your other TF
- Still no luck? Try another TF in the course
(don't forget to check web.bu.edu/aldolase/biochemistry/)
- As a last resort contact Jose & Quinn (biochemlabcoordinators@gmail.com)

Required materials

- Textbook:
 - Tolan, Biochemistry Lab Text, 2nd Edition (BU Bookstore)
- Lab Materials:
 - Safety eyewear
 - A bound research notebook
 - Black or blue pens (**NO pencils or white-out!**)
 - Scientific calculator

Lab syllabus (cont.)

Lab safety

Dress appropriately

- Closed-toed shoes, long pants, t-shirt, long hair tied back
- Safety eyewear worn **at all times**

No food, drink, or consumables of any kind

No cosmetics

Lab gloves go in regular trash cans unless told otherwise

All sharps in designated sharps containers

All hazardous chemicals disposed of appropriately (TFs will advise each week)

Arrive to lab on time with tool kits **before the hour**. Do not arrive late!

Repeat violations will result in dismissal for the day and will negatively impact your compartment grade

Lab syllabus (cont.)

Lab Schedule

Experimental topic	Chapter	Discussion dates	Lab dates	Notebook write-up due at end of lab
Photometric Methods for Protein Determination	1	Sept 8-9	Sept 9-15	
Buffers and Titrations	2	Sept 15-16	Sept 16-22	Chapter 1
Preparation of Crude Enzyme Extract	3A-C	Sept 22-23	Sept 23-29	Chapter 2
Ammonium Sulfate Fractionation & Dialysis	3D-E	Sept 29-30	Sept 30 – Oct 6	
Affinity Chromatography & Concentration of Purified LDH by Ultrafiltration	3F-G	Oct 6-7	Oct 7-10, 14 ^①	
Using the Molecular Modeling Program “O” ^②	11A-B	Oct 14 ^③	Oct 15-21	
Functional Characteristics of LDH	4	Oct 20-21	Oct 22-28	Chapter 3 & 11
Gel Filtration; Native M _r Determination	5A	Oct 27-28	Oct 29 – Nov 4	Chapter 4
Polyacrylamide Gel Electrophoresis using SDS; Subunit M _r Determination	5B	Nov 3-4	Nov 5-11	
Native Zone Electrophoresis; “Zymograms”	5C	Nov 10-11	Nov 12-18	
Preparation of Plasmid DNA & Estimation of DNA Concentration	6A-B	Nov 17-18	Nov 19-24, Dec 2 ^④	Chapter 5
Restriction Mapping	6C	Dec 1-2	Dec 3-9	
			Dec 11	Chapter 6 ^⑤

Lab syllabus (cont.)

Pre-laboratory write-up

Complete this every week before lab

A complete pre-lab write-up entry for full credit includes a:

- Title
- Date
- Introduction
 - Three to four sentences about the purpose of your experiments and your objectives for the day
- Procedure including prepopulated data tables and required flowcharts
 - Write down **in your own words** (**not verbatim from the manual**) a protocol for the assays and experiments you will accomplish for the day
 - Check the notebook section in your lab manual to find out which tables and flowcharts you'll need for lab

Meet with your lab partner(s) beforehand to better organize your time in lab

Preparation is key in this course!

Lab syllabus (cont.)

Attendance and Absences

Discussion and lab attendance is **mandatory**

Making up lab work

- Anticipated absences (e.g. jury duty, BU athletics, interviews)
 - Notify lab TF ASAP! Give no less than a week of notice (**must be cleared by Jose & Quinn!*)
- Sudden emergencies (e.g. family death, hospitalization, accident)
 - Requires bona fide documentation (e.g. funeral program, physician's notice, police report)

Quizzes

No make-up quizzes for discussion are available past the discussion date

If you anticipate missing a discussion, you must contact Jose & Quinn more than a week in advance to take a different quiz

Lab syllabus (cont.)

Grading

Laboratory Write-ups

- Entries in pencil, loose notebook pages, and multiple pages stacked and stapled/taped/attached/folded together will **not** be graded.
- A 20% deduction penalty will be applied for each day your notebook is turned in late (**every 24 hours from time due!*)

Discussion Quizzes

- Quizzes given at the end of each discussion section.
- Read introduction and procedure sections in lab manual to prepare for quizzes
- **Turn in your Chapter 1 quiz to your TFs in lab for credit**

Comportment

- Attendance, punctuality, cleanliness, preparation, effort, laboratory skills, quality of experimentation, ability to work in a group, and adherence to safety regulations will all be factored into this evaluation at the end of the semester.

Lab Exam

- The lab exam will be held on the last week of discussion (**Dec 8 & 9**).

Lab syllabus (cont.)

Academic Conduct

Violations in Academic Conduct

- Photocopying from lab manual or discussion slides
- Copying from lab partner, current students, or previous students (any part of the notebook)
- Copying verbatim from lab manual (introduction and lab procedures)

1st offense = zero for assignment; referral to the Dean

2nd offense = failure of course; referral to the Dean

Students are free to talk about data and experiments, but required to work independently on notebook write-ups

Your laboratory manual

In each chapter:

Introduction

- Concepts, assays, and experiments in the chapter are explained

Problems

- While these problems are for your own benefit and will not be graded, you are highly encouraged to do these on your own. These practice problems will help with calculations for the *Notebook* section.

References

- A list of references from the *Introduction* section

Experimental Procedures

- A step-by-step explanation on how to perform the experiments

Notebook

- Use this section for completing your pre-lab and post-lab write-ups

Set-up and Materials

- A list of reagents, materials, and equipment you will need

Appendix

- Answers to the *Problems* section as well as other useful information to help you complete your notebook write-ups

Your laboratory manual (cont.)

Bring your lab manual to discussion

- Ask questions about unclear concepts and procedures

Do not bring the lab manual to lab

- Your pre-lab write-up should be your guide in lab. The lab manual will only act as a handicap.

Read the complete introduction to the lab manual

Read the chapter introduction and procedure sections before each week of discussion to prepare for quiz

As you progress through the lab manual after a few weeks, you will notice the *Experimental Procedures* section becomes less “cookbook” and more of what a real protocol will look like. **It is your responsibility to figure out how to set up your tables, charts, master mixes, flowcharts, and data organization.**

Your laboratory notebook

For each lab week:

Before lab

- Read the introduction and procedure section
- Complete your pre-lab write-up
 - Read the notebook section of the lab manual to find out which tables and/or flowcharts are needed

During lab

- Record all of your findings for all assays and experiments
- Use your time wisely and efficiently

Before leaving lab

- Ensure that you and your lab partner(s) each have all the data needed
- Turn in your lab notebook if it is due for that week

After lab

- Complete your post-lab notebook write-up

Your laboratory notebook (cont.)

For a complete notebook write-up, each week you must have:

Pre-lab write-up

- Title, date, introduction, procedure and any necessary tables and flowcharts

Data Collection

- Show filled data tables and experiment recordings
- You are responsible to having all of your data in your notebook. Graphs and charts based off of missing data will receive no credit and be grounds for academic misconduct.

Notebook Section

- Complete each prompt and exercise, including *Questions*, from the **Notebook** section for each week in the lab manual

Conclusions

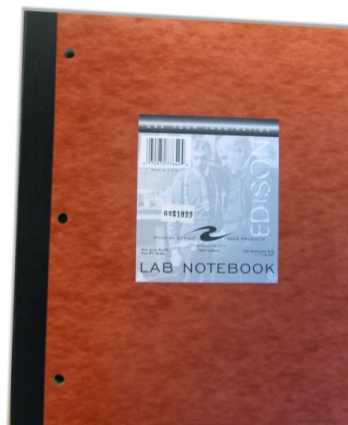
- One or two sentences about the results of your experiments + complete analysis

Sample Calculations

- Show a sample calculation for each different type of mathematical manipulation
- Credit will not be given to values with no sample calculations and are grounds for academic misconduct.

Your laboratory notebook (cont.)

It is your responsibility to keep and maintain professional bound notebook throughout the course!



- Black or blue pen **only**
 - Strike through mistakes
 - **No white-out, no pencils**
- Tape pages neatly to notebook if you have to (**no loose sheets**)
 - Don't stack pages: no flip books/accordions

Chapter 1: Photometric Methods for Protein Determination

Objectives

- Learn two colorimetric methods to measure protein concentration
- Generate a standard curve and observe effect of interfering substances
- Observe absorbance spectra of different macromolecules

Procedures

- Use Lowry Method and Dye-Binding assay to determine protein concentration
- Use UV/Vis spectrophotometer to detect nucleic acids and amino acids

Beer's Law

$$A = -\log T = \varepsilon \cdot l \cdot c$$

$$A = \varepsilon \cdot l \cdot c$$

where,

A is the absorbance value (no units)

ε is the extinction coefficient ($\text{M}^{-1} \text{cm}^{-1}$; value varies for different samples)

l is the path length of the cuvette or glass tube (cm)

c is the concentration of the sample (M)

Your plastic cuvettes have a path length of **1 cm**

Become familiar with Beer's Law. It will come up in subsequent chapters

Protein assays

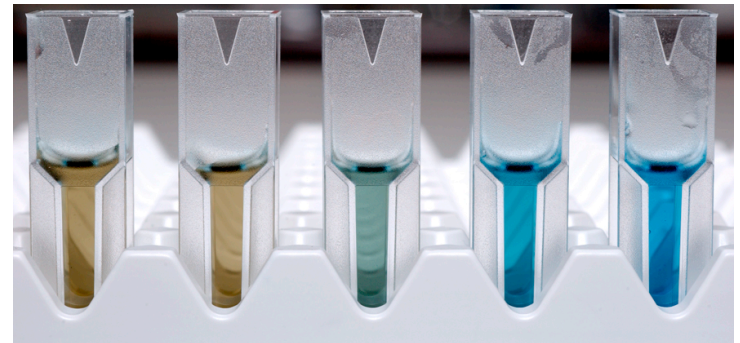
Colorimetric methods (destructive)

Lowry Method

- Sensitive to differences in **time** (stay consistent when adding reagents)
- Method is a combination of **two reactions**
 - Biuret Reagent: alkaline solution of copper ions
 - Turn pink/purple when exposed to protein backbone
 - Cupric Reagent
 - Dependent upon aromatic amino acids

Dye-Binding (Bradford) Assay

- Utilizes the dye Coomassie Brilliant Blue G250, absorbs light at 465 nm
- Upon binding proteins, dye absorbs light at **595 nm**
- Some reagents act as interfering substances and affect absorbance
 - You will test the effects of some interfering substances



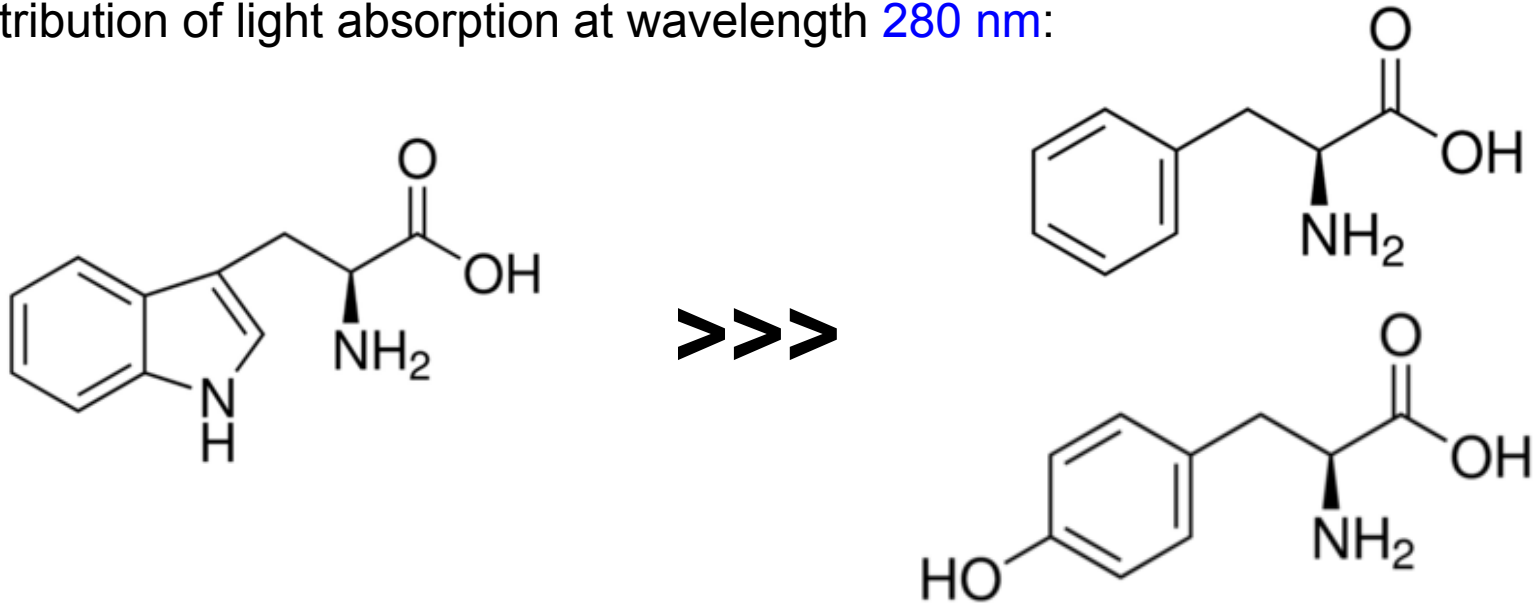
[Protein]

Protein assays (cont.)

Ultraviolet Absorbance (non-destructive)

Proteins absorb light at 280 nm, primarily because of tryptophan residues, and to a lesser extent, phenylalanine and tyrosine

Contribution of light absorption at wavelength 280 nm:



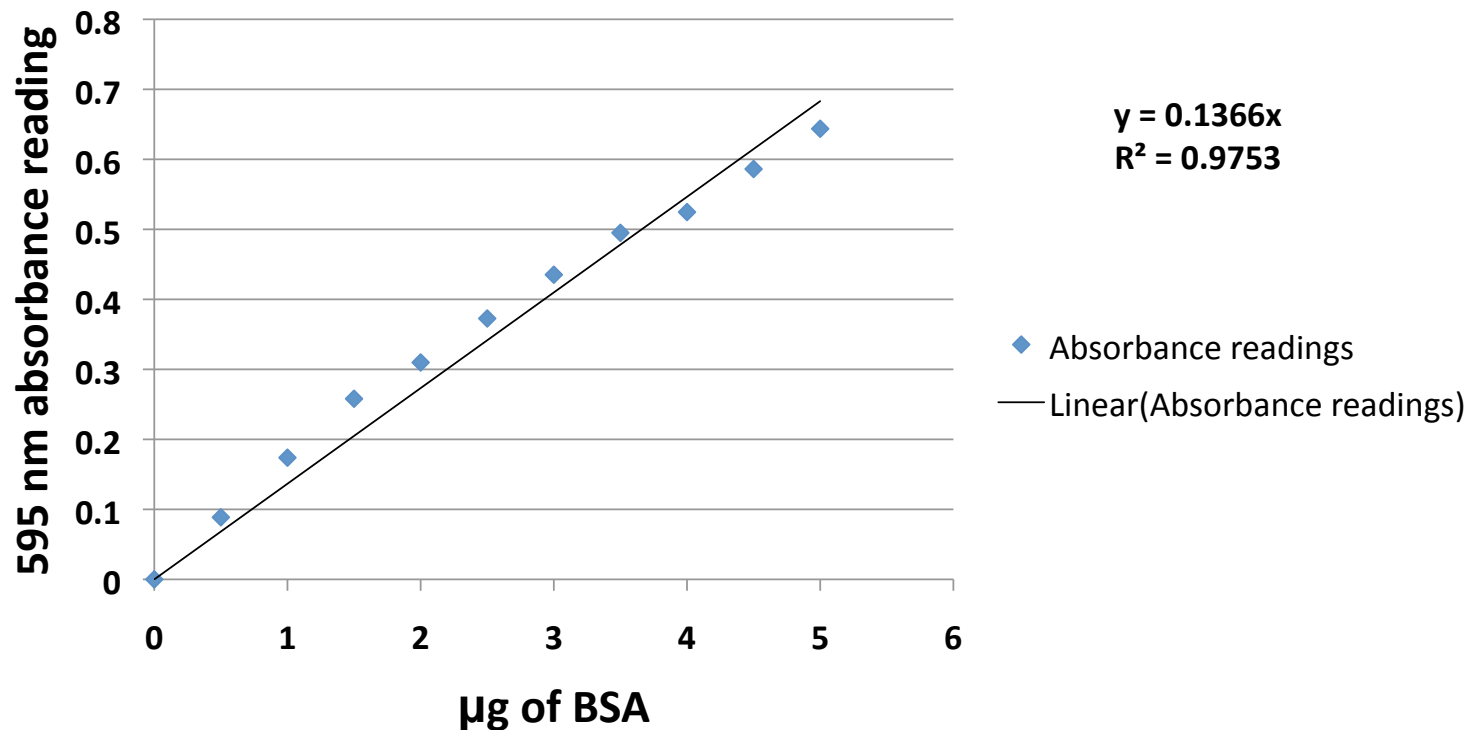
It is difficult to create a standard curve for a heterogeneous sample of protein since amino acid composition varies among proteins

Standard curve

“A plot of the absorbance *versus* the mass or moles of a standard material being assayed” -[page 13](#) of manual

Tubes 1 – 8 for both the Lowry Method and the Dye-Binding Assay will allow you to generate a standard curve for each respective procedure.

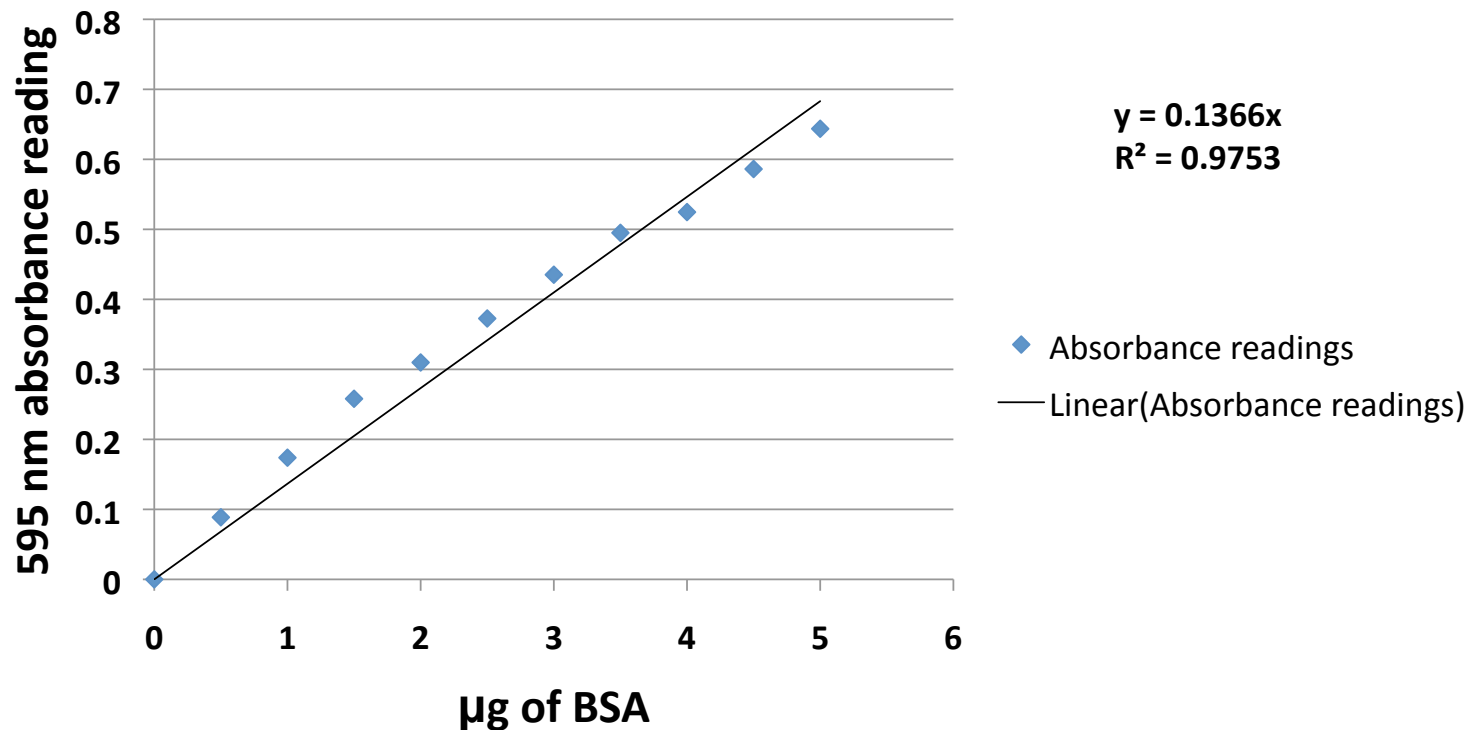
Sample standard curve



Standard curve (cont.)

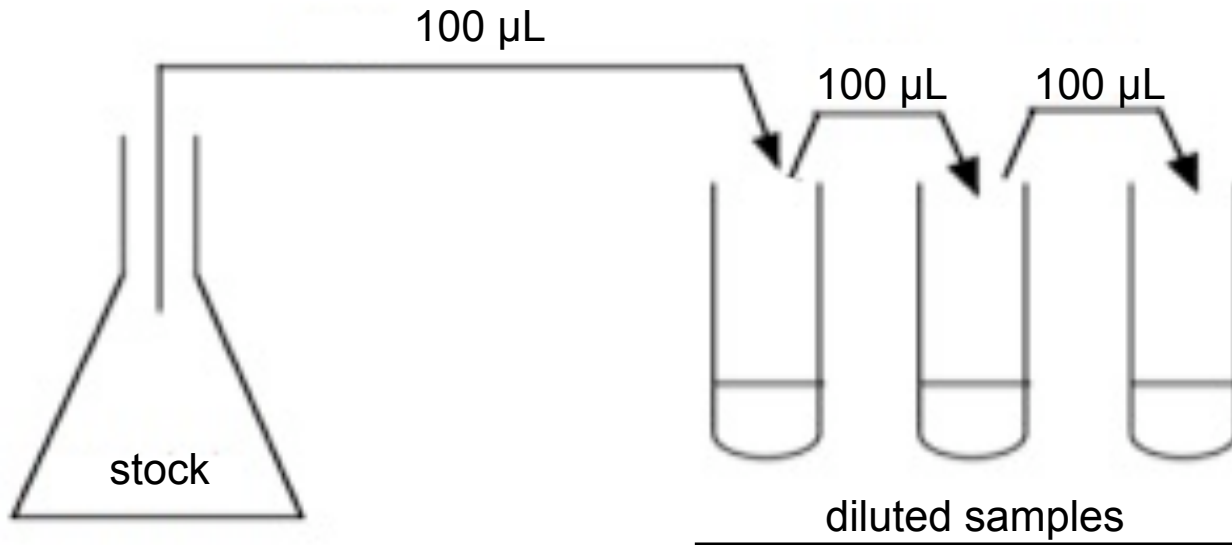
- Interpolate from curve, do not extrapolate. Dilute protein samples and re-assay to land within standard curve data set.
- Make sure linear equation intercepts (0,0) and R^2 is at least 0.95
- After obtaining mass of protein in sample, work backwards to figure out concentration (*i.e.* divide sample mass by sample volume to get concentration)

Sample standard curve



Serial dilutions

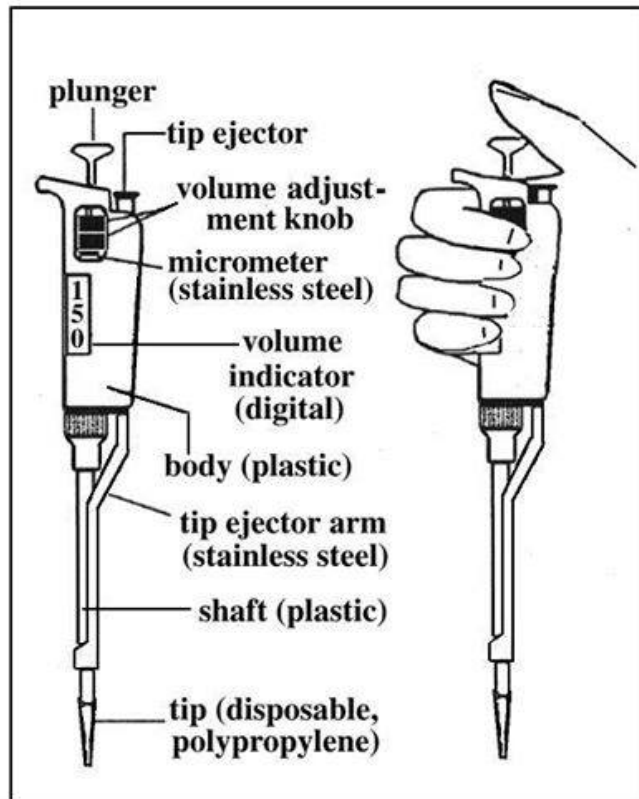
Calculate ahead of time how you will perform your serial dilutions for this week





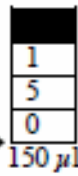


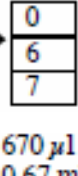



Tube no.	1	2	3
Water volume	900 μL	900 μL	900 μL
Sample volume added	100 μL	100 μL	100 μL
Dilution factor	1/10	1/100	1/1000

Micropipettes

TFs will demonstrate how to properly use and care for micropipettes



Size	Range	Top view and Color	Example Setting	Tip size and color	Tip sample
P-20	2-20 μ l	 yellow	 17.8 μ l	medium yellow	
P-200	20-200 μ l	 yellow	 150 μ l	medium yellow	
P-1000	200-1000 μ l	 blue	 670 μ l or 0.67 ml	large blue	

Chapter 1: Procedure

Lowry Method

- Gather reagents needed & label test tubes
- Prepare each tube according to columns 2, 3, and 4; [page 23](#) in manual
- [This method is time-sensitive! Stay consistent time-wise when adding reagents to tubes \(e.g. space 10 seconds in between each tube\)](#)
- For each sample: add 5.0 mL of alkaline copper reagent (**reagent A**) and immediately vortex
- Let samples stand for 10 min at room temperature
- For each sample: vortex, add 0.5 mL of phosphotungstate-phosphomolybdate (**reagent B**), and vortex again
- Let samples stand for 45 min at room temperature
- Set spectrophotometer wavelength to **550 nm** and blank with tube no. 1
- Read absorbance at **550 nm** and record readings in notebook

Chapter 1: Procedure

Bradford Assay

- Gather reagents needed & label tubes
- Prepare each tube according to columns 2, 3, and 4; [page 24](#) in manual
- Add 4.5 mL of **Blue-Dye reagent** and vortex each tube
- Let samples stand for 2 min at room temperature
- Set spectrophotometer wavelength to **595 nm** and blank with tube no. 1
- Read absorbance at **595 nm** and record readings in notebook

Chapter 1: Procedure

UV-Vis

- [Your TFs will demonstrate how to properly use the UV/Vis spec. Always ask questions if you are unsure about something to avoid damaging/breaking expensive equipment!](#)
- Use the plastic cuvettes in your plastic boxes
- For your UV-Vis session, make sure to blank with 1.0 mL of dH₂O
- Measure absorbance spectra for 1.0 mL samples of BSA (1.0 mg/mL), gelatin (1.0 mg/mL), and yeast RNA (25 µg/mL)
- Print out an absorbance spectra for you and your lab partner(s)

Chapter 1 laboratory checklist

At the end of lab, you should have:

- ✓ Recordings for your Lowry Method readings
- ✓ Recordings for your Dye-Binding Method readings
- ✓ A printout of your UV/Vis absorbance spectra
- ✓ Turned in your completed Chapter 1 discussion quiz to your TFs

For the first week of lab...

- Go to lab first (SCI 162). No need for tool kit yet. Don't be late!
- TFs will introduce themselves, take attendance, and escort you to stockroom
- Check-out tool kit and supply box, then head back to lab
- TFs will go over lab policies, lab safety, and answer questions
- TFs will preview Chapter 1 (**record any procedural changes in your notebook**)
- Complete Chapter 1 experiments

Questions?