Student Instructions and Data for Virtual Western Blot Experiment

Protein concentration determination data:

[BSA standard] (ug/uL)	A_{595} (AU)
0	0.57
50	0.66
100	0.74
250	0.97
500	1.28
750	1.57
beef extract 1:40 dilution	1.21
turkey extract 1:40 dilution	1.16

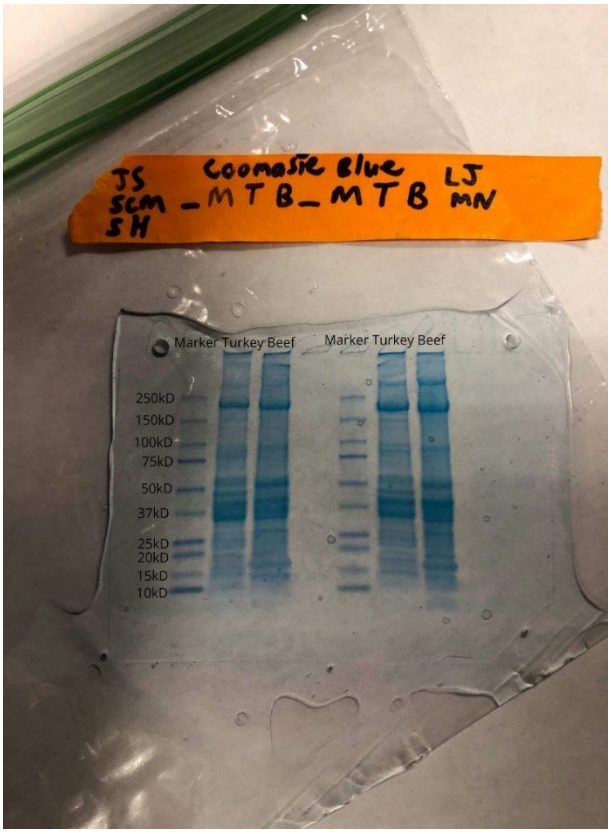
To label, understand, and interpret the images provided to you below:

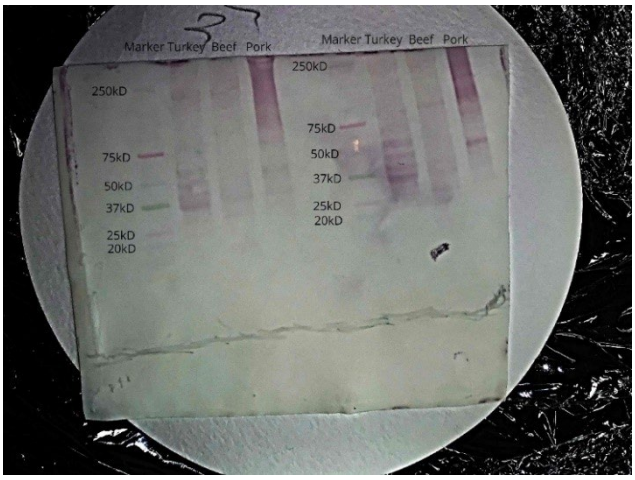
The molecular weight markers are Kaleidoscope prestained molecular weight markers (Bio-Rad catalog number 1610375EDU). Look up the size of each colored band on the Bio-Rad website!

10 micrograms of total protein extract from beef, chicken, or pork was loaded into each gel lane

Order of samples loaded on gels
from left to right: marker, turkey, beef, pork, blank, marker, turkey, beef, pork
(samples from two different lab groups on each gel)

Raw images for you to turn into Figures in your report:





Adjustments and tips for this lab report:

Title: mention species, proteins, methods succinctly

Authors: you only—no need to cite your Professor!

Methods:
verbal explanation of interpolation procedure you used to determine the crude extract protein concentration, including equations and dilutions
explanation of interpolation for size of beta tubulin based on migration on blot
note any deviations from lab manual procedures
include an in-text citation for the lab manual here

Figures:
Please format them according to the instructions and the sample Figures I handed out
Include appropriate labeling--can do this in Powerpoint and then group the image with labels and copy it into Word

- graph for Bradford assay standard curves
- image of Coomassie-stained gel
- image(s) of Western blot(s)
- graph of protein size (kD) (logarithmic axis) versus migration (mm) (nonlogarithmic axis) for your Kaleidoscope standards, derived from Western blot images, on semilog graph paper (you can use the graph paper in your lab manual, or you can adjust the settings on Excel to get it to produce a semilog graph).

Results:
make sure you refer to the results in past tense
use Figure callouts
include text to describe what you see in the Figures

Discussion:
include Figure callouts
make sure you note any anomalies
make sure you try to explain any anomalies
relate your experiment to textbook--or even better, to relevant primary literature sources
suggest specific future experiments

References:
you MUST cite your lab manual and any relevant parts of our textbook to earn full credit

Summary Questions: Look in your lab manual, and in the videos, for the answers to these.

1. How does the Bradford Assay work?
2. What is the purpose/function of the SDS in SDS-PAGE?
3. What is the purpose/function of beta-mercaptoethanol or dithiothreitol?
4. Why does one go through the process of transferring proteins from a gel on to a membrane for Western Blotting?
5. How are monoclonal antibodies produced?
6. What is the purpose of the secondary antibody?
7. What information can a Western blot give that agglutination and precipitation and immunocytochemistry assays cannot give?
8. What similarities and differences, if any, did you observe between the bovine and turkey tubulins?
9. Prepare a graph of molecular weight versus migration for the Kaleidoscope protein markers. Then use that graph to extrapolate the molecular weight of the tubulin proteins in the beef and chicken samples.
10. Is the molecular weight of tubulin in your samples consistent with what is in the literature? If not—provide some possible explanations for any discrepancy.