

Overview of Module 2

1. Name one protein function:

2. What is the purpose of a Western blot?

3. What is the purpose of a BCA Assay? Which metal is present in BCA reagent?

4. What is the purpose of SDS-PAGE?

5. Why do we need to transfer proteins from a gel to a membrane?

Practice Preparing a BCA Assay

1. Calculate serial dilution volumes. We will start with a 20 ug/mL BSA solution.

- a. Need 25 uL of standard for each well x 3 replicates = 75 uL per standard
- b. Always make a little extra: _____ uL per standard

BSA Concentration (ug/mL)	BSA Solution Added (uL)	Water Added (uL)	Total Volume (uL)	Final Volume (uL)
2000 ug/mL	160 uL of 2000 ug/mL	0	160	160 - 80 = 80
1000 ug/mL	80 uL of 2000 ug/mL	80	160	160 - ____ = ____
500 ug/mL	____ uL of 1000 ug/mL	____	160	160 - ____ = ____
250 ug/mL	____ uL of 500 ug/mL	____	160	160 - ____ = ____
50 ug/mL	____ uL of 250 ug/mL	____	160	160 - ____ = ____
5 ug/mL	____ uL of 50 ug/mL	____	160	160 - ____ = ____
0 ug/mL	0	160	160	160

2. Design layout for 96 well plate:

- Number of standards _____ x Number of repeats _____ = _____ wells
- Number of samples 2 x Number of repeats 2 = 4 wells
- Total number of wells = Standard wells _____ + Sample wells 4 = _____ wells
- Label the well plate below with standard and sample placement

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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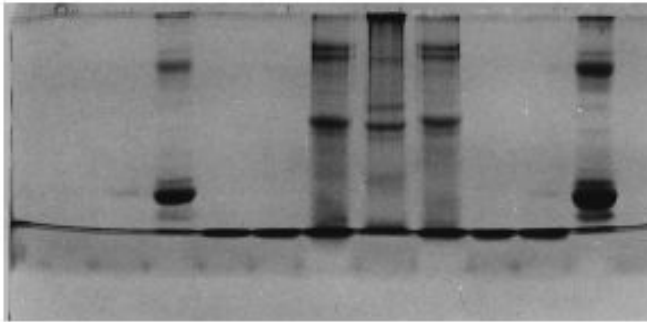
3. Prepare reaction buffer:

- Total number of wells _____ x 200 uL/well = _____ uL reaction buffer
- Always make a little extra: _____ uL
- Combine reagents A, B, and C in ratio 25:24:1 to make reaction buffer
 - Reagent A = (total volume of buffer _____ uL)(25/50) = _____ uL
 - Reagent B = (total volume of buffer _____ uL)(24/50) = _____ uL
 - Reagent C = (total volume of buffer _____ uL)(1/50) = _____ uL

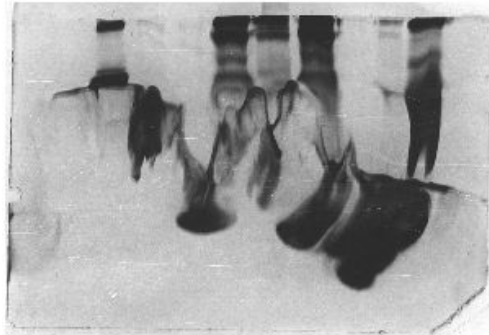
Western Blot Hall of Shame (Challenge Question)

What do you think went wrong in the gels below?

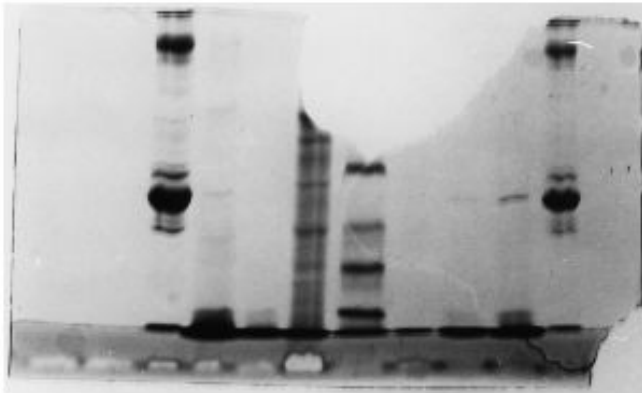
A.



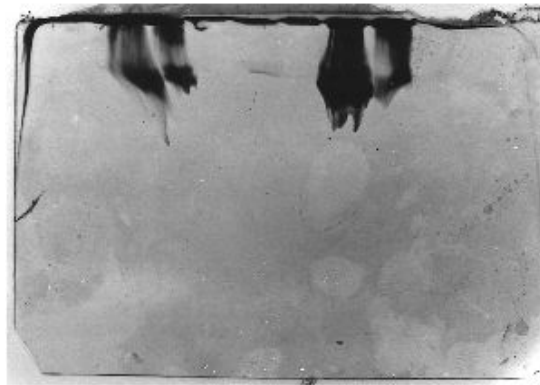
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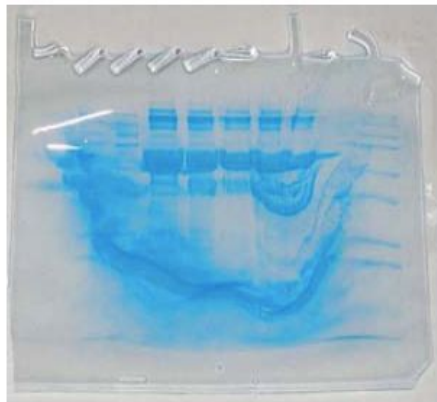
C.



D.



E.



F.

