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:

    a. Number of standards _____ x Number of repeats _____ = _____ wells

    b. Number of samples _2_ x Number of repeats _2_ = _4_ wells

    c. Total number of wells = Standard wells _____ + Sample wells _4_ = ___ wells

    d. Label the well plate below with standard and sample placement

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3. Prepare reaction buffer:

    a. Total number of wells _____ x 200 uL/well = _____ uL reaction buffer

    b. Always make a little extra: _____ uL

    c. Combine reagents A, B, and C in ratio 25:24:1 to make reaction buffer

        i. Reagent A = (total volume of buffer _____ uL)(25/50) = _____ uL

        ii. Reagent B = (total volume of buffer _____ uL)(24/50) = _____ uL

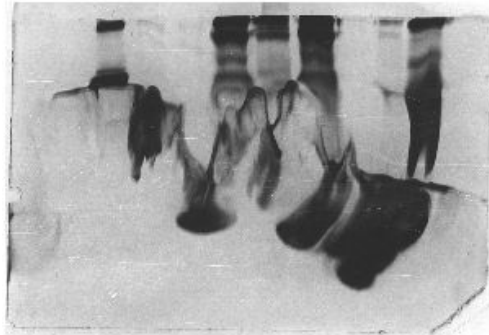   iii. Reagent C =(total volume of buffer _____ uL)(1/50) = _____ uL

Western Blot Hall of Shame (Challenge Question)

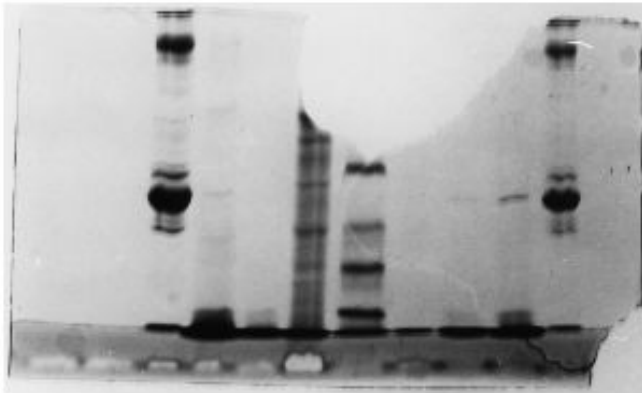What do you think went wrong in the gels below?
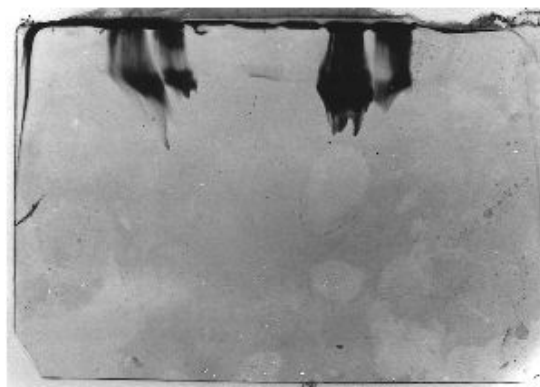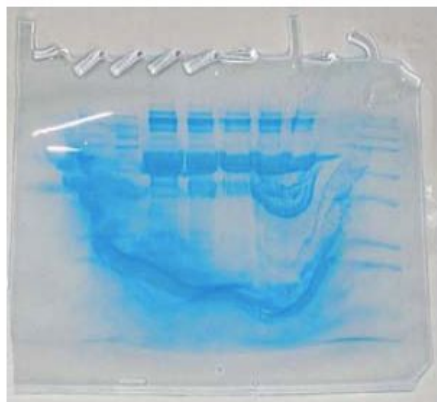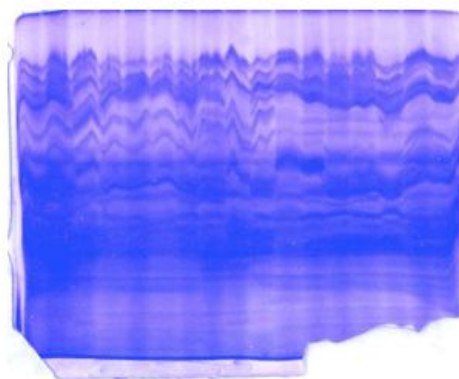
A.
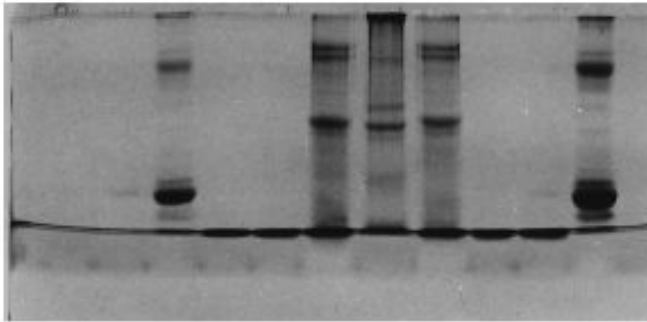
B.

C.

D.

E.

F.

Derivation of Protein Displacement (Challenge Question)

Manipulate Newton's Second Law to demonstrate why smaller proteins travel further than larger proteins.

$$\vec{F} = m\vec{a} = m\frac{d\vec{v}}{dt} = m\frac{d^2\vec{x}}{dt^2}$$